A STUDY OF SERUM ELECTROLYTES ABNORMALITY IN ASTHMATICS

Dissertation

Submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment of the requirements for the award of the degree of

M.D. BIOCHEMISTRY

Branch XIII

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CERTIFICATE

This is to certify that the dissertation entitled "**A STUDY OF SERUM ELECTROLYTES ABNORMALITY IN ASTHMATICS"** is a bonafide work done by **Dr. J. Aaron Vetha Jose** in partial fulfilment of the university rules and regulations for award of **M.D Biochemistry [Branch-XIII]** under my guidance and supervision during the academic year 2013-2016.

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DECLARATION

I Dr. J. Aaron Vetha Jose here by submit the dissertation titled "**A STUDY OF SERUM ELECTROLYTES ABNORMALITY IN ASTHMATICS"** done in partial fulfilment for the award of the degree **M.D Biochemistry [Branch-XIII]** in Sree Mookambika Institute of Medical Sciences, Kulasekharam. This is an original work done by me under the guidance and supervision of Dr.R.Nagendran, M.D.

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LIST OF ABBREVATIONS

GINA	-	Global Initiative for Asthma
NAEPP	-	National Asthma Education and Prevention Program
NHLBI	-	The National Heart, Lung, and Blood Institute
NIH	-	National Institutes of Health
WHO	-	World Health Organization
EPR2	-	Expert Panel Report 2
FEV1	-	Forced expiratory volume in 1 second
PEF	-	Peak expiratory flow
NAC	-	National Asthma Campaign
PAM	-	Pulmonary alveolar macrophages
IL	-	Interleukin
T_H2	-	T helper cell 2
ROS	-	Reactive oxygen species
NO	-	Nitric oxide
IgE	-	Immunoglobulin E
GERD	-	Gastro esophageal reflux disease
NSAID	-	Nonsteroidal anti-inflammatory drugs
cAMP	-	Cyclic adenosine monophosphate
AC	-	Adenyly cyclase
MLCK	-	Myosin light chain kinase
β2AR	-	β_2 agonist receptor
PLC	-	Phospholipase C
MAPK	-	Mitogen activated protein kinase
PKA	-	Protein Kinase A
GRK2	-	G-protein receptor kinase-2
ECF	-	Extracellular fluid
ICF	-	Intracellular fluid
ATP	-	Adenosine triphosphate

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ABSTRACT

Background: Bronchial asthma is characterized by airways inflammation and hyper reactivity of bronchial tissues.

This study was performed on patients with asthma to determine their blood serum electrolyte values and compare them with general healthy population to assess whether there is a difference between them and also to assess the difference between intermediate and persistent asthma groups.

Materials and Methods: This was a case-control study which was performed on patients with asthma attending Sree Mookambika Institute of Medical sciences. Forty-four consecutive volunteer patients with asthma according to the definite criteria where divided into intermittent and persistent asthma groups with twenty two each in each group and healthy sex and age-matched subjects were chosen. Secondary causes of electrolyte disturbances were ruled out. Serum blood sample for measuring sodium, potassium, magnesium, calcium and phosphorus value was obtained.

Results: Significant difference between intermittent and persistent groups was noted ($P<0.001$). Hypomagnesemia and hypokalemia was detected in the asthmatic patients and was significant among the intermittent and persistent asthma groups. Findings of Hyponatremia, Hypocalcemia and Hypophosphatemia were insignificant.

Conclusion: The study showed that asthmatic patients presented with Hyponatremia, Hypokalemia, Hypomagnesaemia, Hypocalcemia or Hypophosphatemia. The association of hypomagnesemia and hypokalemia was seen strongly in asthmatic patients. Between the asthmatic groups serum potassium, serum sodium and serum magnesium of the intermittent asthmatic group is found to be better compared to the persistent group.

Key Words: asthma, electrolytes, intermittent, persistent, hypokalemia, hypomagnesemia

INTRODUCTION

BACKGROUND OF THE PROBLEM

Long standing inflammatory condition of the airways is commonly known as asthma which affects people of all ages. It exerts a sizeable burden on the patients, their families, and the community.[1]It's an illness due to the complementary action between factors that affect at molecular level and the external soundings. The most commonly quoted hostile factors are airborne pollutants which can be indoor or outdoor, high salt intake, indoor allergens, drugs and vaccines.[2]

Respiratory symptoms are characterized with severe attacks that require immediate first aid, if not can lead to death. The burden of asthma is enormous, about 300 million people are currently suffering from asthma worldwide, and about 30 million are living in India.[1,3] Asthma is associated with limitations in day to day activities, absence from school and work days, impairment of lung function, quality of life is reduced, and an unfavourable socioeconomic burden.150 lakh years are lost every year because of asthma, which is approximately 1% of the whole worlds burden of disease.[1]

About 3-38% in children and 2-12% in adults, is currently estimated to be the prevalence of asthma, which is one of the commonest chronic disorder among children.[4] An Indian Study put the prevalence of asthma in India to be around 2.05% among those aged >15 years, with an approximate national burden of 18 million asthmatics.[5]

A large number of asthma patients are over confident about their control level. Exacerbations (67%) have been reported by Indian asthmatics, with a good amount of functional and emotional limitations.[6]This on the whole shows how poorly asthma is controlled and reflects how inadequately treatment is taken up by the patients. The use of bronchodilators, inhaled corticosteroids, and influenza vaccinations is seen in low-income countries like India.[7]

Acidosis and hypoxemia can result due to the use of beta- adrenoceptor agonists and other sympathomimetic bronchodilators, during acute episodes of bronchospasm, which can increase the risk of cardiac arrhythmias.[8]

It's commonly seen and expected that a derangement exists in the serum potassium levels in asthmatic patients undergoing beta 2- agonist therapy.[9,10] The first electrolyte that was found to be deranged and subsequently reported in cases of acute asthma was Hypokalemia and was due to beta 2-agonists and aminophylline therapy.[11,12,13]

The long term implications of using beta 2 -agonists are tremors, tachycardia, palpitation, and anxiety which are commonly seen.[14] Later, asthmatic patients treated with beta 2 agonist were also been reported with hypomagnesemia, hypophosphatemia, and hypocalcemia.[15,16]

The adverse effects of beta 2 -agonists caused while managing acute asthma is one of the main reasons for the increase in mortality rate and which is still on the rise.[17] The use of non-selective beta 2 -agonists (Isoproterenol) and Fenosterol for the treatment of asthma saw increased death incidence in the 1970's.[18] Cardiac

arrhythmias can be precipitated by Hypokalemia, Hypomagnesemia and Hypocalcaemia.[19,20] The possibility of worsening of respiratory failure during acute severe asthma by the impairment of respiratory muscle performance is seen when there is Hypophosphatemia.[21] According to Global Initiative for Asthma (GINA) classification of asthma, Intermittent asthma will have <1symptom per week with <2 nocturnal symptoms per month. In Persistent asthma will have >1 symptoms per week with >2 nocturnal symptoms per month.[1]

There are only few studies related to above subject and its clinical relevance. This study is done to verify the hypothesis that the electrolyte values among the intermittent asthmatic patients will be found to be better than those of persistent asthmatic patients.

AIMS & OBJECTIVES

AIMS AND OBJECTIVES

1. To evaluate serum electrolyte levels in Asthmatic patients.
2. To compare the serum electrolytes (Potassium, Magnesium, Calcium, Phosphate and Sodium) between Intermittent and Persistent asthmatic patients.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Asthma in the past

Towards the end of the 2nd millennium asthma was thought as a disease caused by factors that affect the mind and body. It was considered a hindrance to the advancement of medicine during that period. From the 3rd to 5th decade of the 19th century it was considered as an illness which affects the mind and body together. Being termed psychological, the treatment involved psychoanalysis and "talking cures". Society began to see it as an emotional outcry. Following which asthmatic patients were given medication to heal their depression by psychoanalysts. This idea was later proved wrong and it became a physical disease. Not until the early 1960's Asthma was considered as an inflammatory disease which required anti-inflammatory medications.[22]

Hippocrates Circa 450 BC officially named asthma as a specific respiratory problem leading to the formation of the modern word "panting".[23] It was thought that Asthma was associated with emotions during the early 200BC. [24] As a medical term it was found in the Corpus Hippocraticum written by Hippocrates. He suggested that the asthma spasm usually occurred in fishermen, garment workers and people who work with metal.

Aretaeus of Cappadocia (100AD) who was an ancient Greek clinician gave an observatory report regarding asthma. Asthma was also mentioned several times by Galen (130- 200 AD), who found it to be similar to Hippocrates and

Aretaeus had mentioned about Asthma. He suggested bronchial obstructions the reason for asthma. Asthma originated from the lung pipes was suggested by Van Helmont (1579-1644 AD). A link between asthma and organic dust was noted by Bernardino Ramazzini (1633-1714 AD) along with exercise- induced asthma.[25]

The 1st NAEPP Expert Panel Report was published in 1991, with subsequent updates in 1997, 2002 and 2007 along with NHLBI which contained Guidelines for the Diagnosis and Management of Asthma. GINA along with NHLBI, NIH, and WHO published a Global Strategy for Asthma Management and Prevention, which gave directions towards asthma treatment.

Classification of Asthma Severity

A decision making group along with NAEPP put forth a report known as the EPR2. It provided guidelines to assess symptoms of asthma and pulmonary function before any treatment regimen can be started. They considered parameters like pulmonary function and time of day and night as to when the symptoms came to evaluate the severity of asthma. [26]

Method of EPR 2 for Asthma Severity

Severity	Symptoms	Night time Symptoms	Lung Function
Severe persistent	Symptoms which are continuous, Physical activity limited Exacerbations that are frequent	Frequent	≤60% FEV_1/PEF >30% PEF variability
Moderate persistent	symptoms which are daily, use of short-acting beta-agonist daily, activity affecting exacerbations, ≥2/week exacerbations	In a week >1	>60% FEV_1/PEF but <80% >30% PEF variability
Mild persistent	>2/week Symptoms but <1/day, Activity may affect exacerbations	In a month >2	≥80% FEV_1/PEF, 20% to 30% PEF variability
Mild intermittent	≤2/week Symptoms, Between exacerbations Asymptomatic, brief exacerbations.	In a month ≤2	≥80% FEV_1/PEF <20% PEF variability

The NCA in Australia[27] suggested, that asthma severity be evaluated when a person is stable and also to consider previous course in hospital admission and previous exacerbations. The difficulty lies in identifying hih risk people with asthma as the various parameters distinguishing the hih risk and low risk is blurred.

Method of Australian NAC for Asthma Severity

Severity	Wheeze, tightness, cough, dyspnea	Night time symptoms	Symptoms on waking	Admission or emergency visits	Previous life threatening attack	Short-acting beta-agonist use	FEV_1	PEF
Severe	Every day	week >1	week >1	Usually	May have a history	day >3 to 4	<60 %	<80 %
Moderate	Most days	week <1	week <1	Usually not	Usually not	Most days	60% to 80%	80% to 90%
Mild	Occasional	Absent	Absent	Absent	Absent	week <2	>80 %	>90 %

The British Guideline on the Management of Asthma[28] recommended that symptom controls by a person is to be evaluated individually and also to bring the pulmonary function back to normal with full symptom relief is not possible.

They acknowledged the importance of control of symptoms and normal lung function.

The Canadian guidelines for asthma stated that the asthma control was acceptable, using parameters as to when symptoms were present day or night, physical activity being able to perform and the usage of first line of drugs for immediate relief. Asthma severity is graded depending on symptom control and the requirement for medication.[29]

Method of Canadian Consensus for Asthma Severity

Severity	Symptoms	Treatment
Very severe	May be controlled or not well controlled	use of Short-acting beta-agonist, corticosteroids inhalation, Additional inhaled therapy, corticosteroids taken orally
Severe	Well controlled	Use of short-acting beta-agonist, corticosteroids inhalation, Additional inhaled therapy
Moderate	Well controlled	Use of Short-acting beta-agonist, corticosteroids inhaled Low-to-moderate doses, ± Additional therapy
Mild	Well controlled	use occasionally Short-acting beta-agonist, corticosteroids inhaled in low doses
Very mild	Mild-infrequent	rarely use Short-acting beta-agonist

Classification of Asthma Severity: Method of revised GINA (2006)[30]:

Intermittent

less than once a week symptoms

exacerbations are brief

not more than twice a month nocturnal symptoms

≥80% FEV_1 or PEF

<20% PEF or FEV_1 variability

Mild persistent

more than once a week but less than once a day symptoms

activity and sleep affecting exacerbations

more than twice a month nocturnal symptoms

≥80% FEV_1 or PEF

<20–30% PEF or FEV_1 variability

Moderate persistent

daily symptoms

activity and sleep affecting exacerbations

more than once a week nocturnal symptoms

short-acting β_2-agonist inhaled daily

60–80% FEV_1 or PEF

>30% PEF or FEV_1 variability

Severe persistent

daily symptoms

exacerbations are frequent

nocturnal asthma symptoms are frequent

physical activities Limited

≤60% FEV_1 or PEF

>30% PEF or FEV_1 variability

Respiratory Anatomy

The components of the respiratory system are:

- Nose and nasal cavity
- Mouth
- Larynx
- Trachea
- Bronchial tubes
- Lungs
- Muscles of respiration

Two-thirds of the upper thorax is occupied by the lungs. The spine, heart and mediastinum bind it medially and the diaphragm binds it inferiorly. The inner surface of the chest wall and lung is covered by the parietal and visceral pleura which have a sliding contact facilitating free movement for normal breathing.

Inspiration is the respiratory activity in which there is a downward displacement of the diaphragm along with an upward and outward displacement of the ribs. This is made possible when the external intercostal muscles are made to contract. Expiration is a passive respiratory activity due to elastic recoil nature of the lungs.

The alveolar surface is connected to the external environment by means of a conducting airways which extends from the nose to the alveoli. The processes involved on inhalation is initially air filtration by nose, then rising the temperature to about 37^0C with addition of water vapour before being delivered to the alveoli.

Foreign bodies and tumours usually cause obstruction to the glottis and trachea as the total airway cross section is the least. Turbulent airflow in the larynx, trachea and main bronchi result in what we say as normal breath sounds. As we move away from the main bronchi towards the alveoli the air flow is very slow. It is mostly quiet and gas exchange takes place here by diffusion.

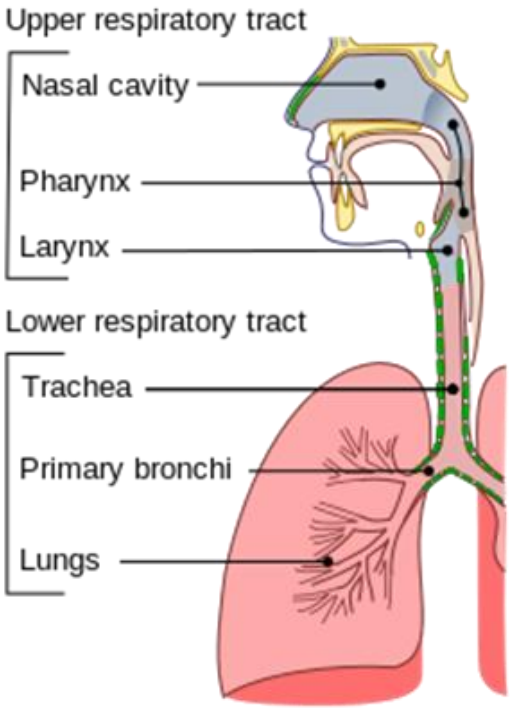


Fig 1: Parts of Respiratory system

The various branches of the respiratory path are

- Trachea
- main bronchus
- lobar bronchus
- segmental bronchus
- conducting bronchiole
- terminal bronchiole
- respiratory bronchiole
- alveolar duct
- alveolar sac
- alveolus

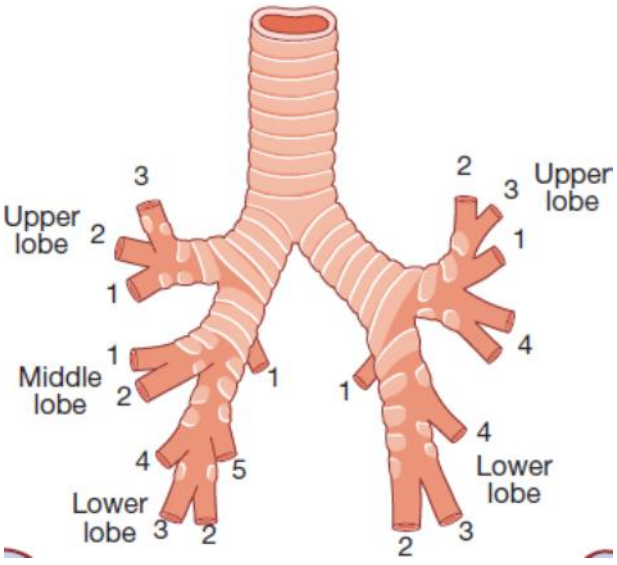


Fig 2: Major Broncho pulmonary segments

The major Broncho pulmonary segments shown in above fig 2 are:

Right

Upper lobe: (1) Anterior,

(2) Posterior,

(3) Apical.

Middle lobe: (1) Lateral,

(2) Medial.

Lower lobe: (1) Apical,

(2) Posterior basal,

(3) Lateral basal,

(4) Anterior basal,

(5) Medial basal.

Left

Upper lobe: (1) Anterior,

(2) Apical,

(3) Posterior,

(4) Lingular.

Lower lobe: (1) Apical,

(2) Posterior basal,

(3) Lateral basal,

(4) Anterior basal

The smallest unit for gas exchange in the lung is the acinus. It consists of branching bronchioles and alveoli. Acinus functions as the meeting place of blood in pulmonary capillaries with air. Here the O2 and CO2 exchange takes place. Type I pneumocytes line the surface of the alveoli. Type II pneumocytes produce surfactant which is a phospholipid mixture preventing the surface tension collapsing the alveoli.[31]

There are three regions which are interconnected to help the flow of air from the external environment to the alveoli namely upper airway, conducting airway and the alveolar airway. The nose and mouth form the entry path which continues into the pharynx where the larynx begins from the lower part forming the upper airway. The air enters through the nose, it is lined with mucosal epithelium which sees a high content of inhaled allergens, toxicants and particulate matter.

The trachea and its branches is the starting point of the conducting airway. The branching causes the increase in lung surface area. The transfer to and from the upper airway is done by the first 16 generations of passage. These are made up of bronchi, bronchioles and terminal bronchioles. The main function is to conduct air to reach the lung. The lamina propria is attached to thin basement membrane above which the mucosal epithelium is attached.

The airways divide 23 times between the trachea and alveolar sacs. The transitional and respiratory bronchioles, alveolar ducts and alveoli found in the last 7 generations form the transitional and respiratory zones. The total cross sectional area at the trachea is 2.5cm^2 whereas at the level of the alveoli it is 11800cm^2. The

airflow velocity is very low at the alveoli. There are about 300 million alveoli in an average human lung with 70cm^2 total area connecting with the pulmonary capillaries.

Special cells are found in the alveoli such as Pulmonary alveolar macrophages, lymphocytes, plasma cells, mast cells & neuroendocrine cells. Pulmonary alveolar macrophages (PAM) function as the defence system of the lung. Its origin lies in the bone marrow. It is actively phagocytic in that it ingests small particles missed out earlier trying to reach the alveoli. Inhaled antigens are also processed by the PAM by secreting molecules that are found to be attracted to granulocytes. At times its functioning is harmful to the human body in the presence of cigarette smoke or irritant leading to the formation of lysosomal products resulting in inflammation.[32]

The cartilaginous, membranous and gas-exchanging bronchi are the various components that form the respiratory bronchioles and the alveolar ducts. The anatomic dead space, is provided by cartilaginous bronchi and membranous bronchi, which also contribute to resistance of the airway. The terminal bronchioles, are approximately 0.5 mm in diameter.[33]

AIRWAY CELL STRUCTURE

The various components of the airway cell structure are:

- Mucosa,
- Basement membrane
- matrix of Smooth-muscle
- fibrocartilaginous or fibroelastic-supporting connective tissue.

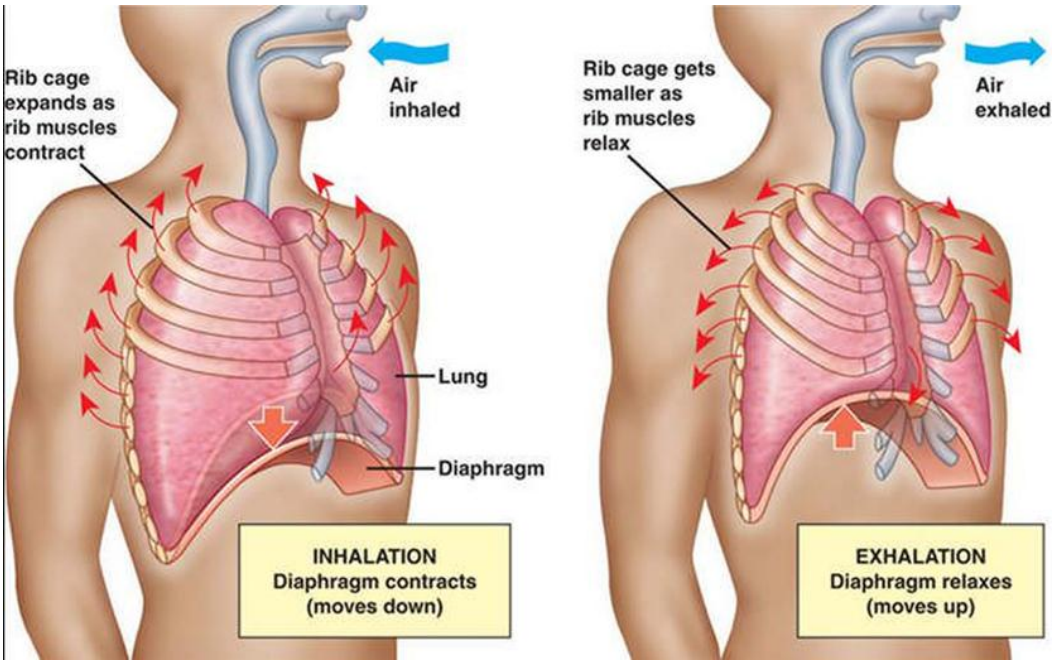


Fig 3: Changes during respiration

Pathophysiology

The major functions of respiration are

(1) Pulmonary ventilation, exchange of air between alveoli and atmosphere,

(2) Diffusion of oxygen and carbon dioxide from and to the alveoli and blood,

(3) Transportation of oxygen and carbon dioxide from and to the body's tissue cells,

(4) Ventilatory regulation.

The movement of the diaphragm upwards and downwards results in the quiet normal breathing. The contraction of the diaphragm results in inspiration and the relaxation of the diaphragm results in expiration causing the lungs to expel air. The main role of pulmonary ventilation is to constantly provide fresh new air to gas exchanging area of the lungs which are found close to the blood vessels of the lung namely the alveoli, its sacs, its ducts, and respiratory bronchioles.

Generally asthma is thought to be due to hypersensitive contraction to some foreign substances which is found in air. Allergic hypersensitivity due in to pollens in the majority of young patients below 30 years where as in adults and old age, hypersensitivity is mostly due to non-allergic substances found in air.

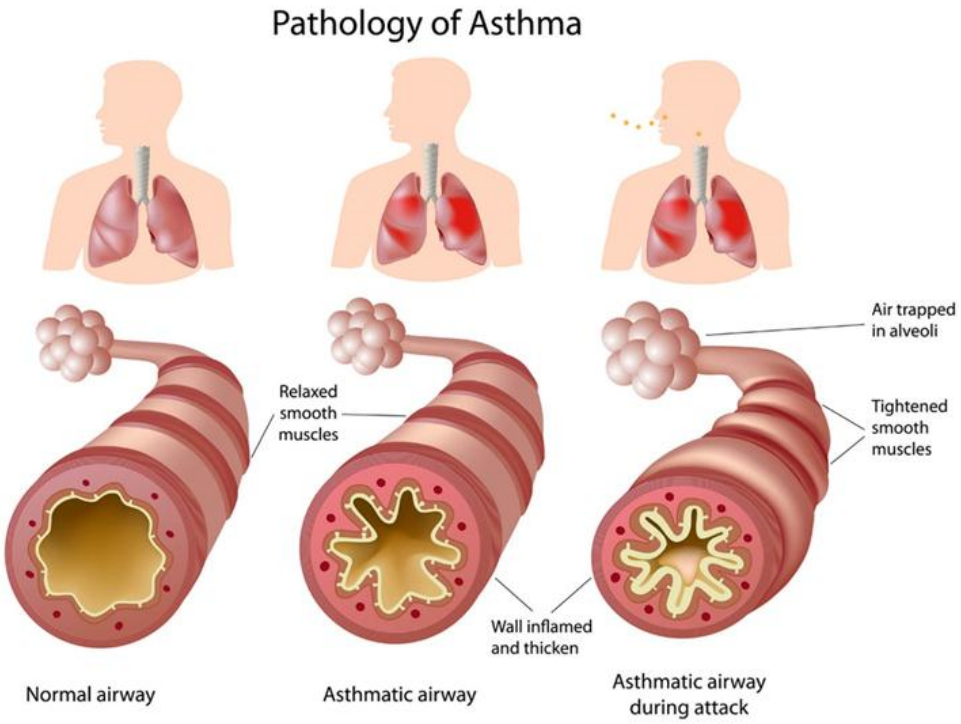


Fig 4: Airways pathology

Mechanism in allergic asthma

Activated eosinophils and T lymphocytes are found throughout the airway mucosa with mucosal mast cells being activated. The disease severity is poorly related to the degree of inflammation. Thickening of basement membrane due to deposition of sub-epithelial collagen is a characteristic finding in asthma. This feature is seen even in those who don't have asthma- those having eosinophilic bronchitis who present with cough. The lumen shows plenty of epithelial cells with the epithelium being very soft and having reduced connections with the airway wall. In fatal asthma the airway is thickened very much and is also edematous.

The mucous plug blocking the airway lumen is a common finding of asthma of fatal nature. The mucous plug is made of glycoproteins from the goblet cells along with plasma proteins which come from bronchial vessels which leak. Increase in the number of blood vessels is also noted along with vasodilatation. The airways being narrow, red and edematous can be seen by direct observation.

INFLAMMATION

The bronchi shows much dominance in inflammation followed by the respiratory mucosa from trachea to terminal bronchioles. Allergic diseases like rhinitis also show a similar characteristic pattern of inflammation when compared with asthma. No single cell can be attributed to the main cause of asthma as there are many kinds of inflammatory cells involved.

Mast Cells

The acute bronchoconstrictor response to allergens and other stimuli is mainly brought about by the mast cells. The other stimuli may include exercise, fog and even hyperventilation. They are mostly found in the smooth muscle layer of the airway. Normal people or patients with eosinophilic cough do not possess mast cells. Allergens with the help of an IgE- dependant mechanism causes the activation of the mast cells. In turn it is bound with specific IgE, making it sensitive to activation.

Mast cells release histamine, cysteinyl-leukotrienes, cytokines, chemokines, growth factors, neurotrophins and other mediators.

Macrophages and Dendritic Cells

Macrophages are obtained from monocytes of the blood. They easily find their way into the asthmatic airways when activated by allergens. Inflammatory response with the release of certain cytokines is mostly due to macrophages. Anti- inflammatory mediators like IL-10 are also produced by such cells. Dendritic cells attach with allergens and process them. The allergens are converted to peptides which are transferred to the local lymph nodes. At the lymph nodes the allergenic peptides react with T- lymphocytes which initiates the creation T cells which are allergen specific.

Eosinophils

Infiltration by eosinophils is another characteristic feature of the airways in an asthmatic. At the time of late reaction, there is a marked increase in activated eosinophils due to the inhalation of allergens. Airway hyperresponsiveness caused due to basic protein release and oxygen derived free radicals is linked to eosinophils. The eosinophils are attached to the vascular endothelial cells of the airway circulation which is caused by the interaction between the adhesion molecules. These with the help of chemokines move into the submucosa. Patients who are not asthmatic but are said to have chronic cough also show features of eosinophilic inflammation. They are also considered to be involved with release of growth factors which are needed for remodelling of the airways.

Neutrophils

Patients with severe asthma and during exacerbations show elevated neutrophils count in sputum and the airways.

T Lymphocytes

The release of certain specific patterns of cytokines with a coordinated inflammatory response in asthma is brought about by T lymphocytes. Following which there is recruitment and survival of eosinophil and mast cell population in the airways.

In the normal airways T_H1 cells predominate whereas the naïve immune system and the immune system of asthmatics are changed to express the T_H2 phenotype. T_H2 cells brings about the release of IL-5 which is closely related with eosinophilic inflammation.

Structural Cells

The cells of structure of the airways are cells of epithelium, airway smooth-muscle cells and fibroblasts. In asthma they provide inflammatory mediators like cytokines and lipid mediators.

INFLAMMATORY MEDIATORS

In asthma many mediators have been shown to be the cause and each having varying effects on the airways leading to the asthmatic features. Mediators such as histamine, prostaglandins, and cysteinylleukotrienes contract airway smooth muscle, increase microvascular leakage, increase airway mucus secretion, and attract other inflammatory cells.

Chemokines

Inflammatory cells from the bronchial circulation are attracted into the airways by chemokines.

Oxidative Stress

In asthma the activated inflammatory cells, such as macrophages and eosinophils, produce large number of ROS resulting in increased oxidative stress. Disease severity is related to elevated oxidative stress, which aggravate the response of inflammation and the response to corticosteroids.

Nitric Oxide

Several cells along with NO synthases produce nitric oxide in the airways. The cells mainly involved are the epithelial cells and the macrophages. Patients with asthma normally expire higher levels of nitric oxide. This is closely

associated with eosinophilic inflammation. This might be the reason for bronchial vasodilatation which seen in asthmatics.[34]

Abnormally large amounts of IgE antibodies are formed leading to allergic reactions with specific antigens. In asthmatic persons the antibodies are attached to mast cells which release different substance when a suitable sensitive substance is made contact. The released substances are

(a) Histamine,

(b) Anaphylaxis substance (leukotrienes mixture),

(c) Eosinophilic chemotactic factor, and

(d) Bradykinin.

Airway resistance is produced by the combined effects of the above factors including localized edema of small bronchioles, thick mucus secretion and spasm of smooth muscle.[35]

The various components involved in the pathophysiology of asthma are:

- inflammation of airway
- obstruction of airflow
- hyper responsiveness of Bronchial system

Airway inflammation

The various stages of inflammation in Asthma is acute, sub-acute, or chronic depending upon the mechanism of inflammation. Airflow obstruction and reactivity of bronchial tissue is because of edema of the airways and secretion of mucus. Different stages of eosinophil infiltration, mononuclear cell infiltration, mucus hyper secretion, desquamated epithelium, hyperplasia of smooth muscle with airway remodelling can also be seen.[36]

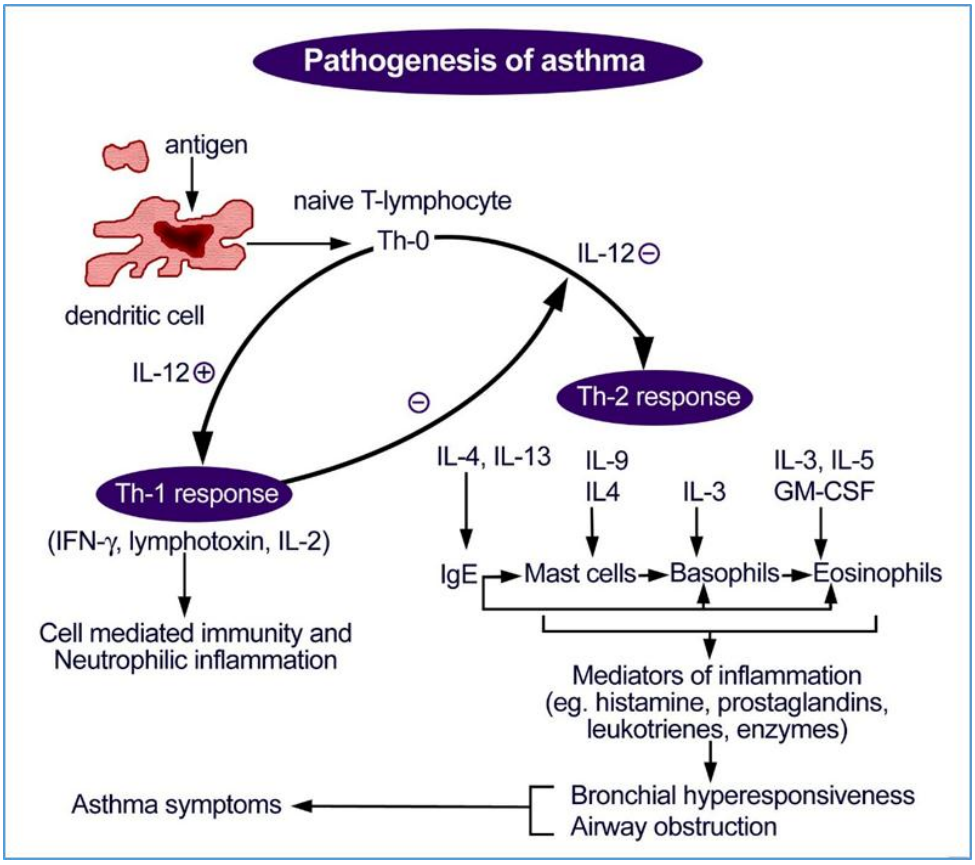


Fig 5: Pathogenesis of asthma

When the airway is inflamed the most common cells that are visualised are macrophages, mast cells, epithelial cells eosinophils and T lymphocytes. T lymphocytes help in the release of numerous cytokines needed to control the

inflammation. The long term duration of the disease is due to endothelial cells, fibroblasts, and epithelial cells. Selectins, integrins, and other similar adhesion molecules help in reducing the inflammation. Lastly, mediators of the cell cause the final change in airway structure which influence smooth muscle tone.

The overstated answer to various internal and external stimuli is seen as hyperresponsiveness of the airway or hyperreactivity bronchial tissues in asthma. The underlying cause is smooth muscle of airways are stimulated directly and gets stimulated indirectly by active substances mast cells or nonmyelinated sensory neurons. The asthma severity is found to be correlating to the response of the airway.

Airflow obstruction

A variety of changes can cause airflow obstruction, which include acute bronchoconstriction, edema of the airway, long standing mucous block formation, and airway cellular restructuring. The initial response in asthma is to constrict the bronchial tissues by the action of IgE dependant mediator release when in the presence of allergen. Later response is the edema of the airway after 6 – 24 hrs following an allergen challenge. The emitted fluid containing serum proteins and cell debris form the long standing mucous plug block which usually takes time to settle. Chronic inflammation leads to changes in the structure of the airway with remodelling of the airway, where by resolvement of the obstruction is hindered for reversibility.[37]

Bronchial hyperresponsiveness

Ventilatory pump failure and hypercapnia are seen as the end result during exacerbations, during the inflammatory process, during the ventilation/perfusion (V′A/Q′) mismatching, and during increased airflow resistance. All of which are seen when the respiratory muscles attain fatigue.[38] By augmenting the activation of the inspiratory muscles an increased inspiratory airflow resistance can be compensated[39], but expiratory flow is further limited by the increased expiratory resistance, seen along with the reduced lung elastic recoil which is present in these patients.[40] This is physiologically much more harmful as expiration is primarily an effort independent, and cannot be compensated by increasing the expiratory muscle effort.[41]

Etiology

The various factors that can cause asthma are:

- allergens from the environment
- Viral infections respiratory tract[42]

- Exercise, hyperventilation[43,44]
- GERD[45]
- Long term sinusitis or rhinitis[46]
- NSAID[47]
- Use of beta-adrenergic receptor blockers (including ophthalmic preparations)
- Obesity[48]
- , tobacco smoke, pollutants of environment
- Occupational exposure[49]
- Irritants (eg, household sprays, paint fumes)
- Various high- and low-molecular-weight compounds (eg, insects, plants, latex, gums, diisocyanates, anhydrides, wood dust, and fluxes; associated with occupational asthma)
- Emotion, stress
- Perinatal factors (prematurity and increased maternal age; maternal smoking and prenatal exposure to tobacco smoke)

Epidemiology

During childhood asthma is mainly seen in boys, then during puberty it is seen to double in girls than boys, followed by which the distribution becomes equal. After puberty it is more prevalent in females, and they dominate the cases

diagnosed in persons older than 40 years. By late adolescence boys tend to have a decrease in symptoms compared to girls. The reason behind such an increased prevalence in young adults and old age is due to limited pulmonary function and response of the airway.[49]

Worldwide about 300 million individuals are affected with asthma. Yearly, 150 lakh years are lost due to the disease burden along with reports of death on a large scale about 250000 is currently been estimated by the World Health Organization (WHO).[30]

A recent study put the prevalence of asthma in India to be around 2.05% among those aged >15 years, with an estimated national burden of 18 million asthmatics.[5] A higher rate of allergic sensitization, decreased lung function, and significantly worse quality of life was noted in older asthmatic adults when compared with controls.[50]

When compared with younger patient groups, elderly patients face a relatively larger morbidity, mortality and expense. The reason being a high number of unplanned outpatient visits, emergency room visits, and asthma-related hospitalizations. The death rate due to asthma for patients older than 65 years is 14 times more than patients aged 18-35 years, once the adult patient is hospitalized.[51-54]

Treatment

The main aspect of treating an asthmatic patient involves the management of acute episodes of asthma while trying to reduce of his/ her long

term symptoms, which include night time symptoms and symptoms arising due to exercise.

Presently asthma is being more successfully treated with anti-inflammatory therapies. About 85% of the patients have their symptoms controlled adequately while using corticosteroids that are inhaled, antagonists of cysteinyl leukotriene receptor and/or anti-IgE Injections. [55]

The ideal goal in treating any patient is to control symptoms, reduce the proportion of sickness when it starts, reduce the proportion of factors that tend to affect mentally and physically thereby give him/ her relief. To begin it is necessary to tackle the causative effects of bronchoconstriction and inflammation. To treat and control asthma symptoms, drugs which affect the adrenergic/ cholinergic bronchial smooth muscle tone as well as drugs which reduce or stop inflammatory processes are used. There is a balance which is maintained between the broncho constrictive effect of the cholinergic system and the broncho dilating effects of the adrenergic system on the bronchiolar smooth muscles in a normal lung. Inhaled short acting β2 adrenergic agonist is commonly used as a quick reliever medication. The controller drugs commonly used are corticosteroids that are inhaled, β2 agonists that are long acting, modifiers of leukotriene, cromolyn sodium and /or methylxanthines. The severity of the patient's disease decides the route and dosage of drug administration.

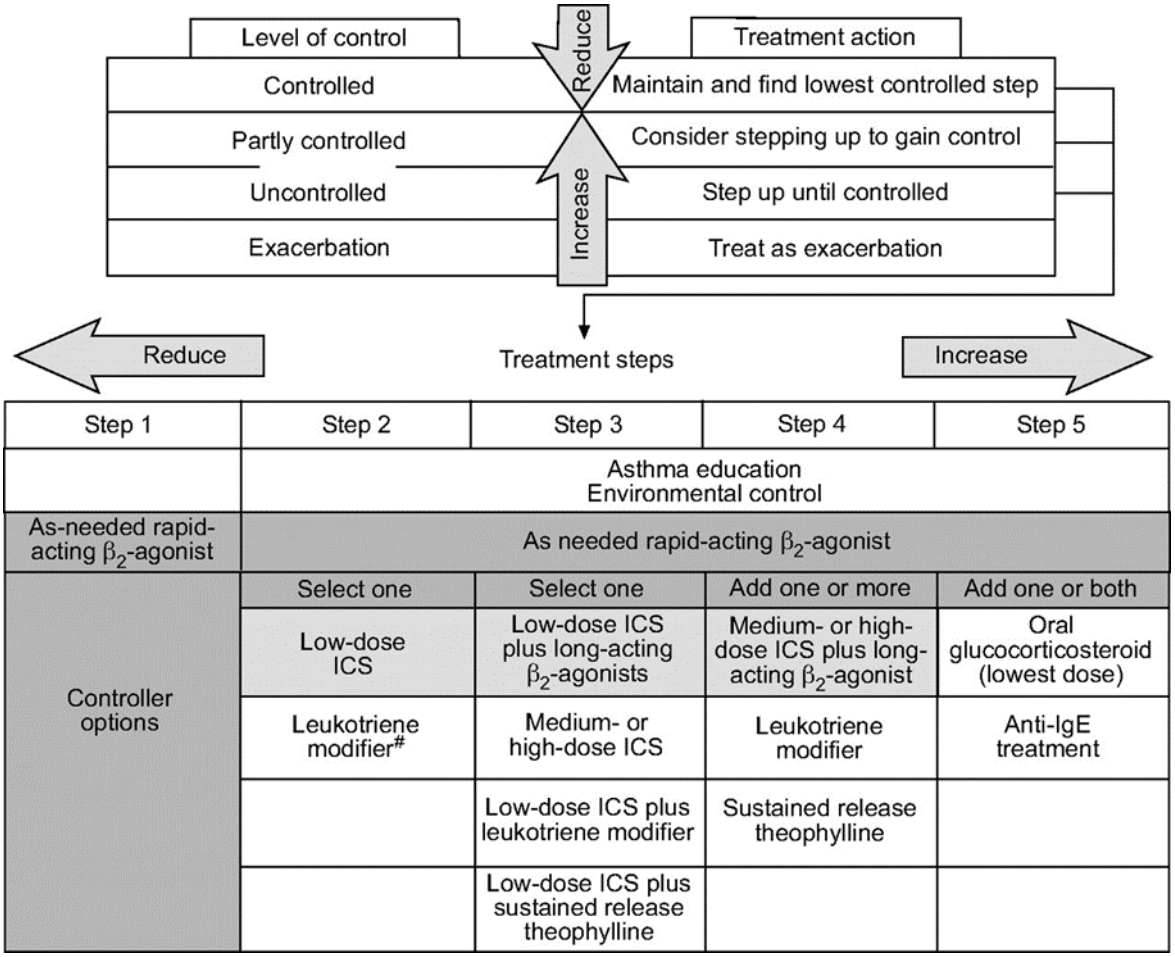


Fig 6: Asthma management

A ladder model is presently being used to serve as a guideline which is divided on the basis of age into 3 groups (0-4 y, 5-11 y, 12 y and older). The clinical manifestations of asthma i.e symptoms, sleep disturbances, daily activity limitations, lung function impairment and use of rescue medications are controlled in many patients with appropriate treatment. When asthma is controlled, there should be no more than occasional recurrence of symptoms and severe exacerbations should be rare.[56]

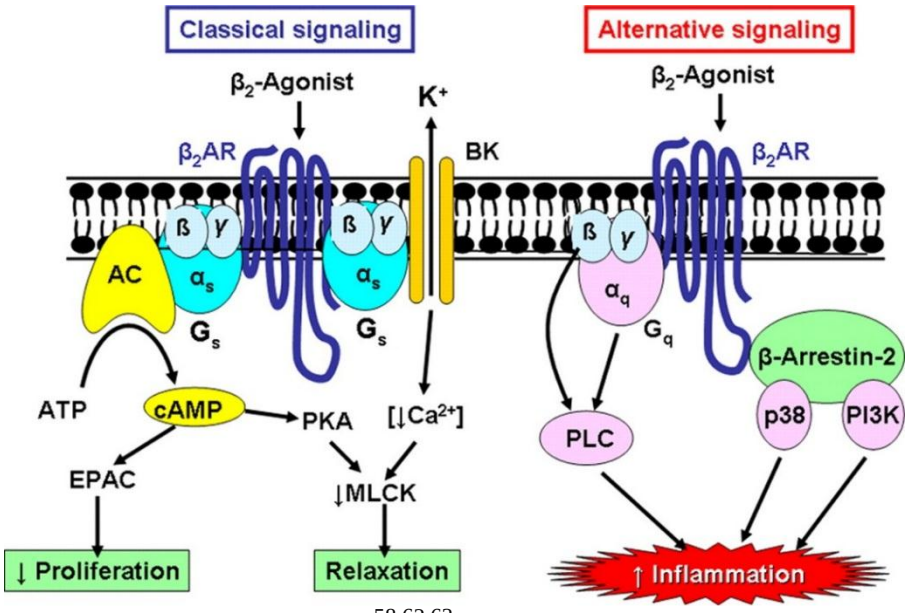
Biochemical aspect of asthma

It is distinguished by an unstable airflow obstruction which is secondary compared to the pattern seen in allergic airway inflammation. In which inflammatory cells like eosinophils and T-helper 2 (T_H2)[57] cells are seen infiltrating and allergens are seen activating resident mast cells and dendritic cells.[57,58] Activated inflammatory cells and inflammatory mediators are produced by the structural cells, such as airway epithelial cells and smooth muscle cells. Increased number of airway smooth muscle cells, fibrosis, angiogenesis, and hyperplasia of mucus-secreting cells are some of the structural changes in the airways due to chronic inflammation. Many cytokines and chemokines along with multiple inflammatory mediators bring about this complex chronic inflammation.[59]

The interaction of cells involved in the human airway tissues such as the lymphocytes, mast cells, basophils, airway epithelial cells and airway smooth muscle were confirmed using murine models and majority found to be true in humans too. [58,60,61]

Routine inspection of the airway is not possible, so airflow obstruction is used as an alternative means for assessing asthmatic airway inflammation. FEV1 is an important component in asthmatic airflow obstruction.[62] Airway inflammation cannot be measured by FEV1 or by any other measures of airflow obstruction.[63] Hence anti-inflammatory drugs along with corticosteroids have been used because of their anti-inflammatory effect. The problem begins when these medications are slowly weaned. A balance between weaning without which can

result in serious harmful side effects on the long run along and the increase in



exacerbations has to be maintained.[58,62,63]

Beta agonist - They are the most effective bronchodilators while treating asthma, as they act as functional antagonists and as well as relax the smooth muscle cells of the airway.[64]

Mechanism of bronchodilation

The mechanism of broncho dilation can be divided into:

- Classical signalling.
- Alternative signalling.

Fig 7: Mechanism of brochodilation

Classical signalling

In the classical signalling the β2 agonist initially binds with the β2 agonist receptor (β2AR). This is then coupled to a stimulatory protein GS. This protein GS combines with Adenyly cyclase (AC) forming cyclic adenosine monophosphate (cAMP) using ATP. The cAMP becomes an exchange protein adenylyl cyclase (EPAC) known to inhibit cell proliferation.[64,65] Meanwhile cAMP can also activate Proteinkinase A along with Calcium ions forming Myosin light chain kinase (MLCK) in the smooth muscle cells of the airway resulting in its relaxation. The MLCK gets inhibited when β2AR couples with GS to a calcium activated potassium channel (BKCa) through which, intercellular calcium is reduced and Myosin light chain phosphokinase is activated.

Alternative signalling

In this signalling, the phospholipase C (PLC) is activated by the β2AR and βγ-subunits of Gα and Gq and via Gαq. β-arrestin-2 is also found to also interact with β2AR, which interacts with p38 (Mitogen activated proteinkinase)MAPK and Phosphoinositide 3-kinase (PI3K). These pathways increase proteins of inflammation which have a harmful effect over the asthma.The airway smooth muscle cell proliferation gets inhibited by β2-agonists which is due to EPAC rather than PKA.[64,66]

Airway the smooth muscle of the airways are usually resistant to β2AR desensitization, because of a large receptor reserve and a very low level of expression of the enzyme G-protein receptor kinase-2 (GRK2). The β2-receptors are inactivated and phosphorylated by GRK2.[67]

An alternative way in which β2 agonist work is by restricting inflammatory cells from producing bronchoconstrictor mediators and airway nerves from producing neurotransmitters. For example, β2-agonists inhibit the release of mediators from mast cell by GS coupled to Ca^{2+}-activated K^{+} channel.[68]

Conditions that mimic bronchial asthma

There are times when the respiratory can produce sounds similar to that of asthma which may be confusing to the clinician. The commonly seen conditions are:

- Vocal cord dysfunction

At times Vocal cord dysfunction may be seen as a single entity or combined with asthma. The reason being - paradoxical adduction of the vocal cords while breathing in, which becomes absent with panting, speech, or laughing.[69]

- Tracheal and bronchial lesions

There are various reports suggesting that airway tumors such as endobronchial carcinoid and mucoepidermoid tumours can also create symptoms just like asthma.[70]

- Foreign bodies

Wheezing as such is known to occur in infants and adults. When a foreign body is aspirated, it causes generalised wheezing or localized wheezing leading to symptoms like asthma.[71]

- Pulmonary migraine

Recurrent asthma, thick mucoid sputum with cough, lower back pain to the shoulder with nausea and vomiting is pulmonary migraine which is associated with focal headaches.[72]

- Congestive heart failure

The feeling of dyspnea and wheezing due to enlarged lung vessels and interstitial pulmonary edema causes congestive heart failure. The presence of wheeze secondary to bronchospasm is seen in cardiac asthma which can give rise to paroxysmal nocturnal dyspnea and coughing.[73]

- Diffuse panbronchiolitis

A condition called diffuse panbrocholitis is known to have symptoms like bronchial asthma along with wheeze, cough, and sinusitis with dyspnoea on exertion.[74]

- Aortic arch anomalies[75]

- Sinus disease[76]
- Gastroesophageal reflux[77]

Electrolytes

Electrolytes are divide into anions that are ions with negative charge which move toward the positive electrode, or cations that are ions with positive charge that move toward a negative electrode. The major electrolytes are Na^+, K^+, Cl^- and HCO_3^- which are found primarily as free ions, compared to Ca^{2+}, Mg^{2+}, and other trace elements which are mainly protein bound.[78]

In a healthy person, 60 % of the body weight is body water. Body water is of two types namely ECF and the ICF, with a cell membrane in between. With the help of Na, K ATPase pump, equilibrium between the two compartments is maintained, where by Na being the main extracellular cation and K the main intra-cellular cation. The capillary membrane divides the ECF into intravascular and interstitial compartments. The membrane pore size, the relative concentration and oncotic pressure of proteins decides the equilibrium between the compartments.[79,80]

Sodium

The major cation of extracellular fluid is Sodium. It maintains the normal water distribution along with osmotic pressure of the ECF. The body requires 1 to 2 mmol/d, over which gets excreted by the kidneys.

Potassium

The major intracellular cation is Potassium. In tissues, the average concentration is 150 mmol/L, where as in the erythrocytes the concentration is 105 mmol/L. An average dietary intake of 2.4 to 4.4 g/d is needed daily to maintain the requirement. Potassium is rapidly distributed after being absorbed from the GI tract, cells take up a small amount, and mostly excreted by the kidneys. After glomeruli filtration it is completely reabsorbed by the proximal tubules and finally secreted in the distal tubules in exchange for Na+ under the influence of aldosterone.

Magnesium

The fourth major cation in the body is Magnesium as well as the second most major intracellular cation. The cellular concentration of magnesium has a range of 2.4 to 7.3 mg/dl. Usually if the cell has more metabolic activity then there would be more magnesium as it is bound to the clleular ATP. Increased nerve conduction velocity with lowered threshold of axonal stimulation is seen when there is low serum magnesium. Neuro muscular excitability is increased due to reduced serum magnesium resulting in various metabolic abnormalities.

Calcium

. Calcium is the most prevalent cation in the body about 1000mg in the free calcium form and is the active biological form. It plays an important role in muscle contraction, hormone secretion, glycogen metabolism, and cell division as

intercellular calcium. Maintains intercellular calcium, bone mineralization, blood coagulation and plasma membrane potential as extracellular calcium. Decreased calcium is known to cause increased neuromuscular excitability and tetany.

Phosphorus

An adult body is said to contain has 600 g of phosphorus in the form of inorganic and organic phosphates. About 10% of the phosphate in serum is protein-bound; 35% is complexed with sodium, calcium, and magnesium; and 55% is free. Being an important component of hydroxyapatite, it plays an important role in body structure.

Conditions causing electrolyte imbalance:

The human body during the time of sickness and disease is known have some sort of electrolyte disturbance. The most common electrolyte disturbances are conditions which arise due to high and low sodium, potassium, calcium and magnesium.[78]

A plasma Na+ concentration <135 mmol/L is said to be Hyponatremia which is seen in conditions such as Hyperlipidemia, Hyperproteinemia, Posttransurethral resection of prostate/bladder tumor, Hyperglycemia, Integumentary loss as in sweating & burns, Gastrointestinal lossas seen in with vomiting, tube drainage, fistula, obstruction, Diarrhea, Renal loss as with diuretics, osmotic diuresis, hypoaldosteronism, salt-wasting nephropathy, post obstructive

diuresis, nonoliguric acute tubular Necrosis, Primary polydipsia, Chronic renal insufficiency, Heart failure, Hepatic cirrhosis and Nephrotic syndrome.[81]

A plasma Na+ concentration >145 mmol/L is Hypernatremia which is seen in primary hypodipsia, loss of water from GI tract, insensible loss of water due to evaporation from skin and respiratory tract, renal water loss due to drugs or diabetes insipidus.

A plasma K+ concentration <3.5 mmol/L, is Hypokalemia which is seen in conditions such as Starvation, Clay ingestion, Metabolic alkalosis, insulin secretion disorder,β_2 adrenergic receptor agonists, α blockers, TPN, Pseudohypokalemia, Hypothermia, Hypokalemic periodic paralysis, Barium toxicity, Gastrointestinal loss (diarrhea), Integumentary loss (sweat), Increased distal flow: diuretics, osmotic diuresis, salt-wasting nephropathies, Increased secretion of potassium as in primary hyperaldosteronism, secondary hyperaldosteronism (malignant hypertension, renin-secreting tumors, renal artery stenosis, hypovolemia), apparent mineralocorticoid excess (licorice, chewing tobacco, carbenoxolone), Congenital adrenal hyperplasia, Cushing's syndrome, Bartter's syndrome, distal delivery of non-reabsorbed anions: vomiting, nasogastric suction, proximal (type 2) renal tubular acidosis, diabetic ketoacidosis, glue-sniffing (toluene abuse) & penicillin derivatives.

A plasma K+ concentration >5.0 mmol/L is Hyperkalemia which is seen in Renal failure, decreased circulating arterial volume, decreased as potassium secretion conditions such as Primary and secondary hypoaldosteronism, and enhanced Cl- reabsorption.

Hypercalcemia is usually seen in excessive PTH production, malignancy, Excessive 1,25$(OH)_2$ D production, Primary increase in bone resorption, Excessive calcium intake and Endocrine disorders such as adrenal insufficiency and pheochromocytoma. Hypocalcaemia is usually seen with Parathyroid agenesis, Parathyroid destruction, Reduced parathyroid function, Vitamin D deficiency, Parathyroid hormone resistance syndromes, Acute pancreatitis and Acute rhabdomyolysis.[82]

A plasma concentration below 1.7 mg/dl is called as hypomagnesemia which can be due to malabsorption from the intestine, vomiting, diarrhea or drainage from the intestine. The rapid movement of magnesium into cells, bone or third spaces from the ECF is also another cause. Hypermagnesemia is usually seen when there is a defect in the renal excretion as normally large quantities of magnesium can be excreted out without any problem.[83]

MATERIALS AND METHODS

SOURCES OF DATA

Patients and bystanders who attended the medicine OPD of Sree Mookambika Institute of Medical Sciences, Kulasekharam, Tamilnadu for routine medical check-up formed the subjects for the present case control study. A total of 88 subjects that came to the hospital were divided into 44 control and 44 cases (22 intermittent and 22 persistent asthmatic cases) during January 2015 to July 2015 was enrolled into the study.

INCLUSION CRITERIA

1. All patients diagnosed with asthma without any comorbidities attending medicine outpatient department and casualty of Sree Mookambika Institute of Medical Sciences grouped into intermittent and persistent depending on their severity of asthma.
2. Age between 18 to 60 years
3. Normal persons without asthma and any other comorbidities.

EXCLUSION CRITERIA

1. Patients excluded were -Cardiovascular disease/ Diabetic/ alcoholic/ chronic kidney diseases/ diarrhoea/ pregnancy/ on diuretics/ drugs producing bronchospasm

2. Those who are not willing to participate.

METHOD OF COLLECTION OF DATA

Informed consent taken from the subjects after explaining the procedure. The data was collected using an organised pro forma. Baseline data including age, gender, occupation, detailed medical history including number of symptoms per week and number of nocturnal symptoms per month, clinical examinations and relevant investigations were included as part of the methodology.

The following parameters were collected: age, gender, religion, blood pressure and clinical chemistry parameters. Blood samples (2ml) were collected in clot activator Vacutainer from each participant while employing standard infection prevention procedures. The serum was obtained after centrifuging the blood for 5 min at 2500 rpm. The serum samples were then used to determine the concentrations of Sodium, Potassium, Magnesium, Calcium and Phosphorus.

1. **ESTIMATION OF SODIUM & POTASSIUM** (Ion selecting electrode method)[84]

Clinical significance

Most metabolic processes are affected by electrolytes. They help to maintain osmotic pressure and various body fluid compartments hydration, pH of the body, and heart and muscle functions regulation. Electrolytes are also involved in oxidation-reduction reactions and participate as essential parts or cofactors in enzyme reactions.

Principle

The Beckman Coulter AU System ISE module for Na^+, K^+, and Cl^- uses crown ether membrane electrodes for sodium and potassium. For a specific ion an electrical potential is developed according to Nernst equation. This electrical potential is compared with internal reference solution and converted in voltage followed with sample ion concentration.

Reagents required:

ISE Buffer		ISE Reference	
Triethanolamine	0.1 mol/L	Potassium Chloride	1.0 mol/L
Preservatives		Preservatives	

ISE Mid Standard		Internal Reference Solution	
Sodium Potassium Chloride Preservatives	4.3 mmol/L 0.13 mmol/L 3.1 mmol/L	Potassium Chloride Silver Chloride Preservatives	3.3 mol/L Saturated

ISE Low Serum Standard		ISE High Serum Standard	
Sodium	130 mmol/L	Sodium	160 mmol/L
Potassium	3.5 mmol/L	Potassium	6.0 mmol/L
Chloride	85 mmol/L	Chloride	120 mmol/L
Preservatives		Preservatives	

Na^+ Selectivity Check Solution		K^+ Selectivity Check Solution	
Sodium	150 mmol/L	Potassium	5.0 mmol/L
Preservatives		Preservatives	

Sample:

- Serum heparinised plasma or EDTA plasma used.
- Specimens should be separated from cells as soon as possible after collection
- Do not use samples with hemolysis may falsely elevate potassium values.
- If plasma must be used, the recommended anticoagulants are lithium heparin and ammonium heparin.

Handling Conditions:

- Use fresh sample to test.
- At 2-8ºC sodium and potassium is stable for 7 days.
- Do not freeze the reagents.
- The samples are stored in a stoppered tube if analysis is delayed.

REFERENCE RANGES:

Serum:

Na^+: 136 – 145 mmol/L

K^+: 3.5 – 5.1 mmol/L

Cl^-: 98 – 107 mmol/L

Preparation:

The reagents are liquid which is readily useable. No extra preparation is needed. ISE electrodes are readymade and can be attached to the on board analyzer.

Storage Requirements

- When stored at 2 – 25^0C, unopened reagents and standards are stable until the date of expiry printed on the label.
- When opened and stored in the ISE reagent compartment of the analyzer. ISE Buffer, ISE Mid-Standard, and ISE Reference Solution are stable for 90 days.
- After opening, ISE Low and High Serum Standards, ISE Urine Low/High Standard, and Na/K Selectivity Check Solutions may be stored at 2 - 25°C for up to 90 days provided the cap is replaced immediately after use.

- ISE Internal Reference Solution may be stored at 15- 25°C for up to 90 days provided the cap is replaced immediately after use.

Precautions:

1. The ISE Reagents and Standards are for in vitro diagnostic use.

2. Not to be ingest. It is harmful if swallowed.

2. **Estimation of Magnesium** (Xylidyl blue method)[85,86]

PRINCIPLE

In an alkaline medium a coloured complex is formed by magnesium when it reacts with Xylidyl blue. With the help of spectrophotometry this colored complex is measured. The calcium interference is removed by the addition of EGTA.

REAGENTS

A. Reagent.

Sodium Carbonate 0.1 mol/L

EGTA 0.1 mmol/L,

Triethanolamine 0.1mol/L,

Potassium Cyanide 7.7 mmol/L,

Sodium Azide 0.95 g/L.

B. Reagent.

Glycine 25 mmol/L,

Xylidyl blue 0.5 mmol/L,

Chloroacetamide 2.6 g/L.

S. Calcium/Magnesium Standard.

Calcium 10 mg/dl,

Magnesium 2 mg/dl (0.82mmol/L).

Aqueous primary standard.

SAMPLES

- Serum collected by following standard techniques of blood collection.
- Hemolysed and lipemic samples are not suitable.
- Serum Magnesium is stable for 10 days at 2-8ºC. Use heparin as anticoagulant.

Reagent reconstitution

Reagents and standard are provided ready to use.

Procedure

The assay was carried out using Au 480 beckmann coulter autoanalyser.

REFERENCE VALUES

Serum and plasma: 1.7-2.4 mg/dl = 0.70-0.98 mmol/L.

STORAGE

Store at 2-8ºC.

Until the expiry date Reagents and Standard are stable when stored tightly closed and if contaminations are prevented during their use.

3. **ESTIMATION OF CALCIUM** (by ARSENAZO III method)[87]

PRINCIPLE

$$\text{Calcium} + \text{Arsenazo III} \xrightarrow[\text{Neutral pH}]{} \text{Blue colored complex}$$

A colored complex is formed when arsenazo III reacts with the calcium present in the sample which is spectrophotometrically measured.

REAGENTS

Reagent.

Arsenazo III 0.2 mmol/L,

imidazole 75 mmol/L.

SAMPLE

- Serum collected by following standard techniques of blood collection.
- Hemolysed and lipemic samples are not used.
- Calcium in serum or plasma is stable for 10 days at 2-8ºC. Anticoagulants other than heparin should not be used.

Procedure

The assay was carried out using Au 480 beckmann coulter autoanalyser.

REFERENCE VALUES

Serum and plasma: 8.6-10.3 mg/dl = 2.15-2.58 mmol/L

REAGENT PREPARATION

Reagent and Standard are ready to use.

STORAGE

Store at 2-8ºC.

Reagent and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

4**. ESTIMATION OF PHOSPHORUS** (by Phosphomolybdate/UV method)[88]

PRINCIPLE

$$\text{Phosphorus} + \text{Ammonium molybdate} \xrightarrow{\text{ACID pH}} \text{Phosphomolybdate complex}$$

In an acid medium, molybdate reacts with inorganic phosphorus to form a phosphomolybdate complex which at 340nm is measured spectrophotometrically.

Reagent

Reagent:

Sulfuric acid 0.36 mol/L,

Sodium chloride 154 mmol/L,

Ammonium molybdate 3.5 mmol/L

Preservatives

SAMPLE

Serum collected by following standard techniques of blood collection. Phosphorus in serum or plasma is stable for 7 days at 2-8ºC.

PROCEDURE

The assay was carried out using Au 480 beckmann coulter autoanalyser.

REFERENCE VALUES

Serum: Adults: 2.5-4.5 mg/dl

REAGENT PREPARATION

Reagent and Standard are ready to use.

STORAGE

Store at 15-30ºC.

Reagents and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

STATISTICAL ANALYSIS

Data collected were entered in excel. Percentage, Mean and Standard deviation were calculated. Appropriate test of significance like independent students “t” test and Fisher’s exact test were done. Values were considered statistically significant if $p < 0.05$.

RESULTS

RESULTS

The present study was undertaken to evaluate the significance of possible electrolyte abnormality in asthmatic patients. 44 Asthmatic patients divided into 2 groups of 22 each intermittent and persistent were considered with 44 healthy individuals chosen as controls.

GENERAL CHARACTERISTICS OF THE STUDY POPULATION

5.1 AGE

Table 5.1

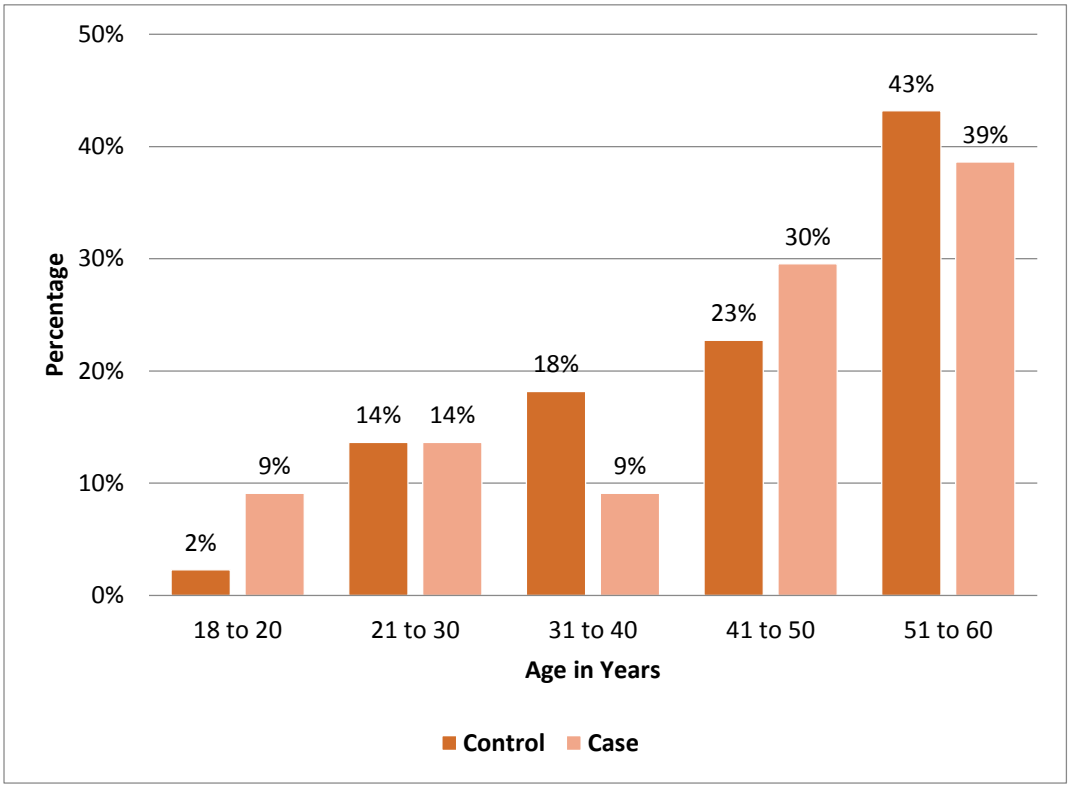
Age	Control		Case	
	Number	Percentage	Number	Percentage
18 to 20 years	1	2	4	9
21 to 30 years	6	14	6	14
31 to 40 years	8	18	4	9
41 to 50 years	10	23	13	30
51 to 60 years	19	43	17	38
Total	44	100	44	100

Table 5.2

Age	Number	Mean	Std. Deviation
Control	44	44.56	12.53
Intermittent	22	43.59	14.83
Persistent	22	43.59	12.9

Table 5.1, gives the distribution of the study samples according to the age and graphically represented in fig 8. The cases and controls are divided into 5 groups (18 -20years, 21-30yrs, 31-40yrs, 41-50yrs, and 51-60yrs). It shows the maximum numbers of controls are in the age group of 51-60yrs (43%) and the majority number of cases is also of 51-60yrs (38%). Table 5.2 gives the mean age of the control group as 44.56 years and that of the intermittent and persistent cases as 43.59 yrs. The age of the youngest person who participated in this study is 18 years and the oldest is 60 years with the mean age of 44.1 years.

Fig 8: Age distribution



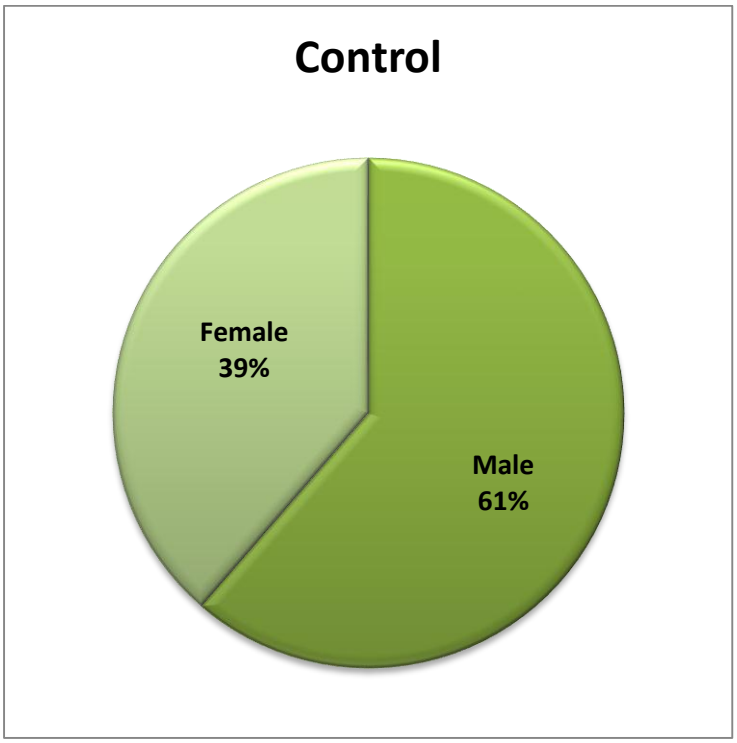
5.2 GENDER

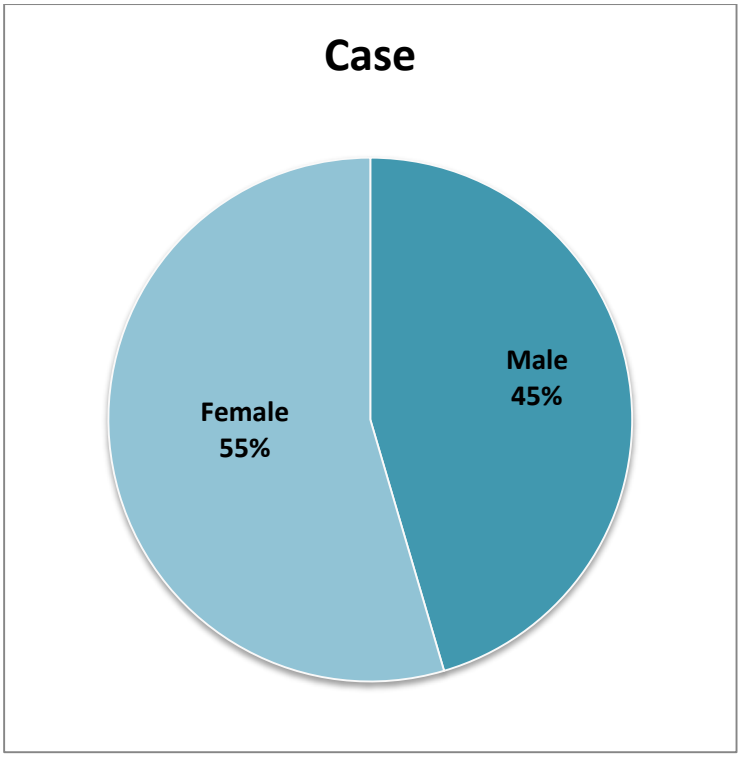
Table 5.2 Sex Distribution

Sex	Control		Case	
	Number	Percentage	Number	Percentage
Male	27	61	20	45
Female	17	39	24	55
Total	44	100	44	100

Out of 44 persons who participated in this study as control, 27 were males (61%), followed by 17 females (45.3%) whereas females were more in the case group with 24 females (55%) followed by males at 20 with 45%.

Fig 9: Sex distribution





5.3 Distribution of Electrolyte parameters

Table 5.3

	Hyponatremia		Hypokalemia		Hypomagnesemia		Hypocalcaemia		Hypophosphatemia	
	n	%	n	%	n	%	n	%	n	%
Control (44)	13	29	0	0	1	0	21	95	0	0
Intermittent (22)	18	81	12	54	22	100	22	100	22	100
Persistent (22)	21	95	21	95	22	100	20	90	21	95

Table 5.3 shows the general distribution of the abnormal electrolytes between the cases and the control group. 95% of the persistent group is said to have Hyponatremia followed by the intermittent and control group. Persistent group shows high distribution 95% for Hypokalemia, followed by intermittent with 0% for control. 100% is seen in both intermittent and persistent group. About 90% of the study population is seen to have hypocalcaemia. 100% hypophosphatemia is seen in the intermittent group followed by 95% in persistent group and nil in control.

5.4 Comparison of serum Sodium between controls and cases

Comparison of serum Sodium between controls and cases is shown in table 5.4-1, 2, 3 and graphically represented in fig 10. The difference in mean Serum sodium levels between (intermittent and persistent) cases and controls is statistically significant ($P<0.05$). More over the difference in mean Serum sodium levels between intermittent and persistent cases is also statistically significant.

Table 5.4-1

	N	Mean	Standard Deviation (±)	P value
Control	44	136.54	2.56	

Case -I	22	134.63	0.9	0.001

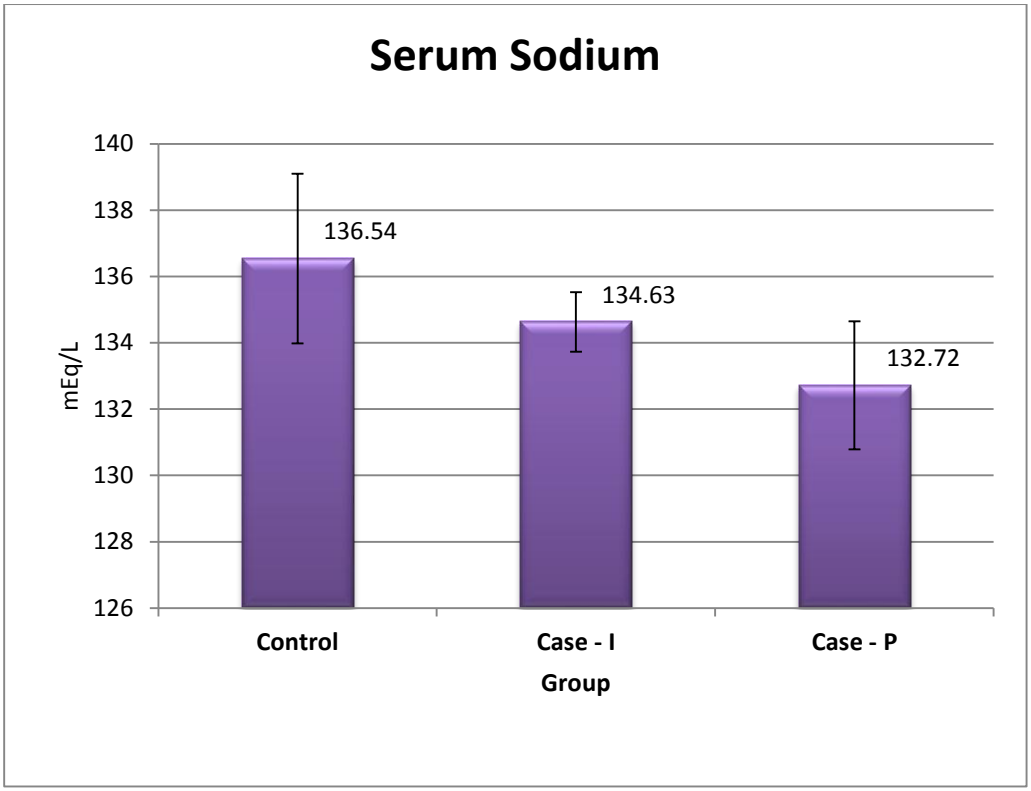
Table 5.4-2

	N	Mean	Standard Deviation (±)	P value
Control	44	136.54	2.56	
Case - P	22	132.72	1.93	<0.0001

Table 5.4-3

	N	Mean	Standard Deviation (±)	P value
Case - I	22	134.63	0.9	
Case - P	22	132.72	1.93	<0.0001

Fig 10: Comparison of mean serum Sodium



5.5 Comparison of serum Potassium between controls and cases

Comparison of serum Potassium between controls and cases is shown in table 5.5-1, 2, 3 and graphically represented in fig 11. The difference in mean Serum Potassium levels between (intermittent and persistent) cases and controls is statistically significant ($P<0.05$). More over the difference in mean Serum Potassium levels between intermittent and persistent cases is also statistically significant.

Table 5.5-1

	N	Mean	Standard Deviation (±)	P value
Control	44	4.37	0.53	
Case - I	22	3.89	0.89	0.008

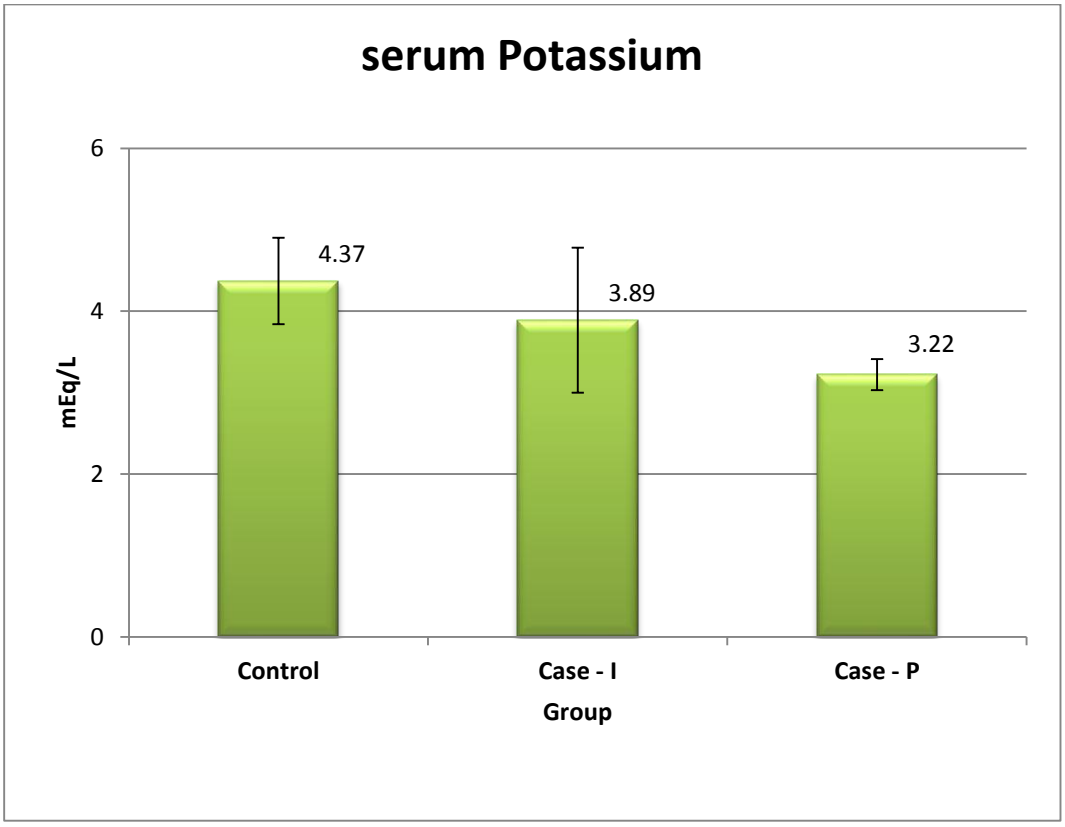
Table 5.5-2

	N	Mean	Standard Deviation (±)	P value
Control	44	4.37	0.53	
Case – P	22	3.22	0.19	<0.0001

Table 5.5-3

	N	Mean	Standard Deviation (±)	P value
Case - I	22	3.89	0.89	
Case - P	22	3.22	0.19	0.001

Fig 11: Comparison of mean serum Potassium



5.6 Comparison of serum Magnesium between controls and cases

Comparison of serum Magnesium between controls and cases is shown in table 5.6-1, 2, 3 and graphically represented in fig 12. The difference in mean Serum Magnesium levels between (intermittent and persistent) cases and controls is statistically significant ($P<0.05$). But the difference in mean Serum Magnesium levels among intermittent and persistent cases is not statistically significant.

Table 5.6-1

	N	Mean	Standard Deviation (±)	P value
Control	44	1.96	0.22	
Case - I	22	1.44	0.15	<0.0001

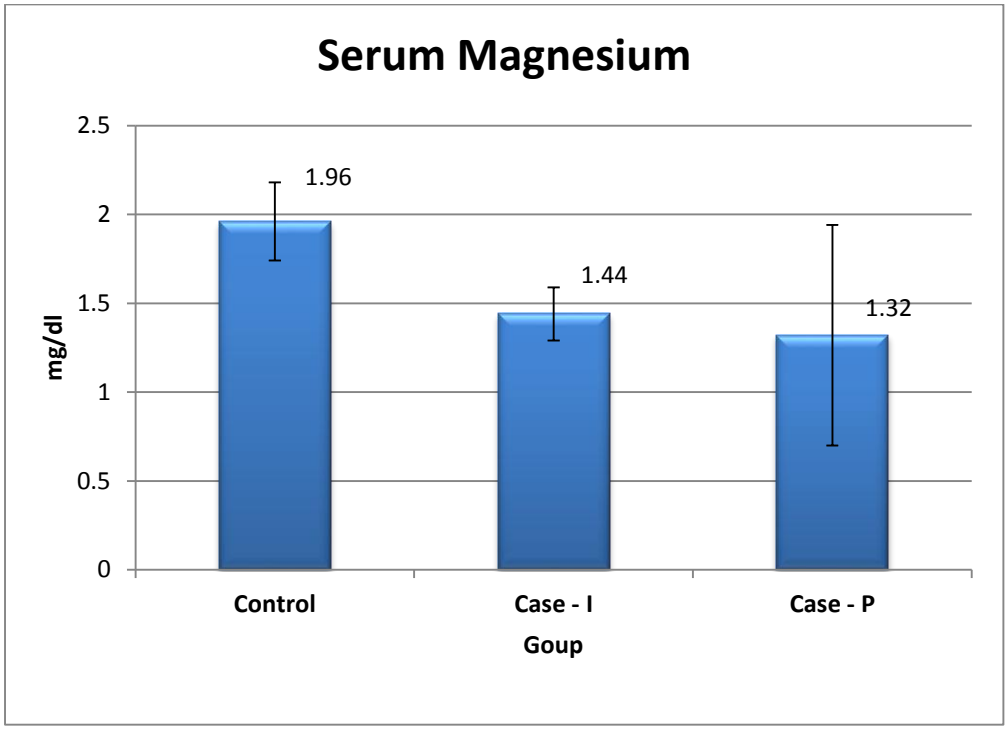
Table 5.6-2

	N	Mean	Standard Deviation (±)	P value
Control	44	1.96	0.22	
Case - P	22	1.32	0.62	<0.0001

Table 5.6-3

	N	Mean	Standard Deviation (±)	P value
Case - I	22	1.44	0.15	
Case - P	22	1.32	0.62	0.392

Fig 12: Comparison of mean Serum Magnesium



5.7 Comparison of serum Calcium between controls and cases

Comparison of serum Calcium between controls and cases is shown in table 5.7-1, 2, 3 and graphically represented in fig 13. The difference in mean Serum Calcium levels between (intermittent and persistent) cases and controls is statistically significant (P>0.05). The difference in mean Serum Calcium levels between intermittent and persistent cases is not statistically significant.

Table 5.7-1

	N	Mean	Standard Deviation (±)	P value
Control	44	8.55	0.69	
Case -I	22	7.7	0.67	<0.0001

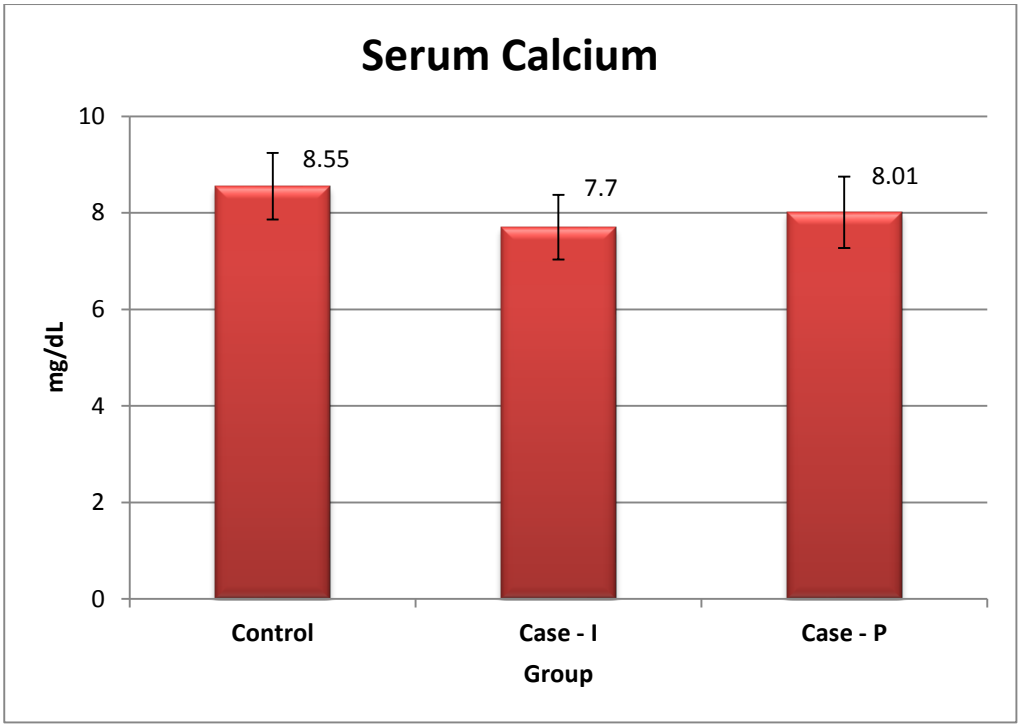
Table 5.7-2

	N	Mean	Standard Deviation (±)	P value
Control	44	8.55	0.69	
Case - P	22	8.01	0.74	0.005

Table 5.7-3

	N	Mean	Standard Deviation (±)	P value
Case - I	22	7.7	0.67	
Case - P	22	8.01	0.74	0.152

Fig 13: Comparison of mean serum Calcium



5.8 Comparison of serum Phosphorus between controls and cases

Comparison of serum Phosphorus between controls and cases is shown in table 5.8-1, 2, 3 and graphically represented in fig 14. The difference in mean Serum Phosphorus levels between (intermittent) cases and controls is statistically significant ($P<0.05$). The difference in mean Serum sodium levels between intermittent and persistent cases is not statistically significant.

Table 5.8-1

	N	Mean	Standard Deviation (±)	P value
Control	44	4.24	0.57	
Case - I	22	2.29	0.1	<0.001

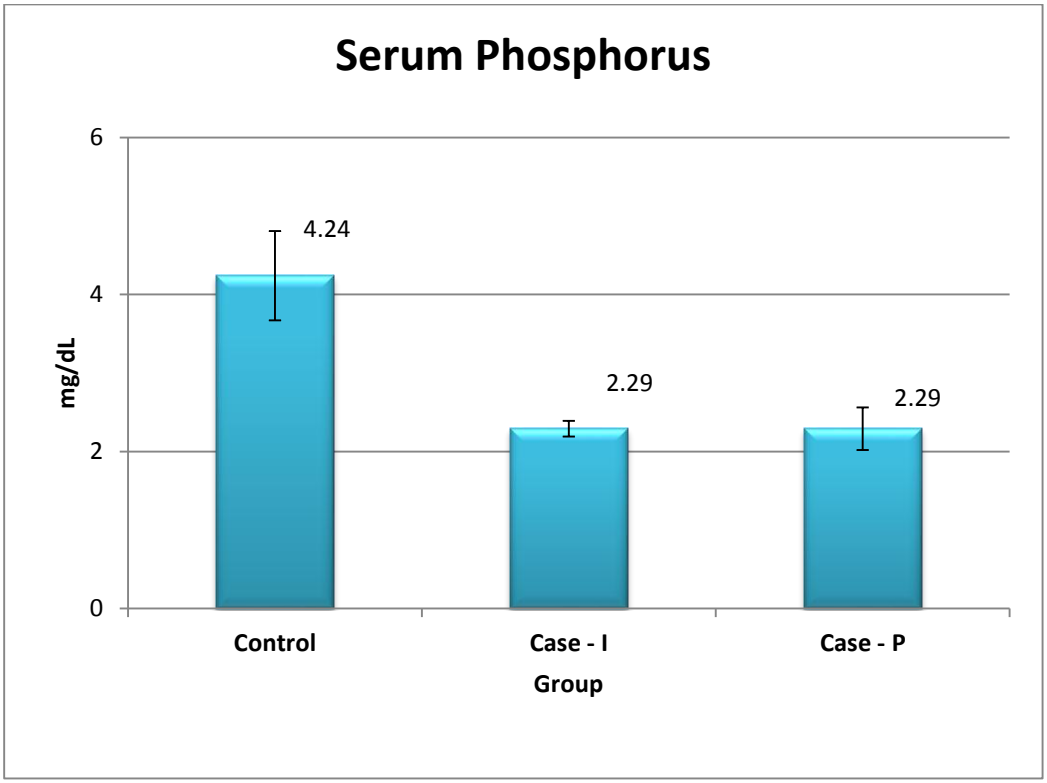
Table 5.8-2

	N	Mean	Standard Deviation (±)	P value
Control	44	4.24	0.57	
Case - P	22	2.29	0.27	<0.001

Table 5.8-3

	N	Mean	Standard Deviation (±)	P value
Case - I	22	2.29	0.1	
Case - P	22	2.29	0.27	1

Fig 14: Comparison of mean serum Phosphorus



5.9 Distribution of electrolytes between cases

Table 5.9

	Hyponatremia		Hypokalemia		Hypomagnesemia		Hypocalcaemia		Hypophosphatemia	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Intermittent	18	4	14	8	22	0	22	0	22	0
Persistent	21	1	21	1	22	0	20	2	21	1
p-value	0.345		0.021		N/A		0.488		1	

significance	NS	S	S	NS	NS

Table 5.9 shows the distribution of electrolytes between the intermittent and persistent asthmatic groups. The distribution of hypernatremia, hypocalcaemia and hypophosphatemia between the intermittent and persistent group was found to be insignificant. The distribution of Hypokalemia and hypomagnesemia is found to be significant.

Discussion:

Bronchial asthma is one of the most common respiratory disorder that can start at any age and is seen to affect children as well as adults. It is a complex disorder that is characterized with episodes of breathlessness, wheeze and cough. The cough might be seen usually only at night or after an intensive exercise or exposure to an irritant. All the symptoms may be due to multiple factors producing responses differently in different individuals. The individual may have some respite from the symptoms in between episodes of bronchial attack. Although bronchial asthma is produced by multiple factors, they are all share one common

thing- the ability to cause inflammation of the airways leading to airway obstruction further leading to difficulty to breathe normally.

Remission is seen during the early childhood (puberty). Zein JG et al in their study reported that age is a risk factor for the severity of asthma between 18yrs to 45yrs. implying that the severity of asthma will be less during early childhood compared to those who become symptomatic during adulthood.[90]

The GINA classification serves as a basic guide line to treat asthma symptomatically rather than cure it completely. Treatment may include beta agonists, cortico steroids which may be inhaled or taken orally to relive the symptoms. Early detection and proper medication can help in decreasing the severity of the disease. This study was done to evaluate the electrolyte abnormality in asthmatic patients with a possibility of noting a difference in electrolyte levels among the persistent and intermittent asthmatic cases.

The mean values of Serum sodium of intermittent and persistent asthmatic patients is 134.63± 0.9 mEq/L and 132.72 ± 1.93 mEq/L respectively. Both the mean values of serum Sodium are found to be lower than the control mean 136.54± 2.56 mEq/L. The mean of serum Sodium in intermittent patients is closer to the control mean stating it is better than the persistent patient's serum sodium. Statistically the difference in mean values were found to be significant, that is hyponatremia seen in intermittent and persistent groups with respect to the control group. The association between the two groups intermittent and persistent was found to be insignificant. The reference range being Na^+: 136 – 145 mEq/L. The reason for hyponatremia could be the use of theophylline which can cause an

elevated excretion of electrolytes and water suggested in the study done by Amin R.[91]

The mean values of Serum potassium of intermittent and persistent asthmatic patients is 3.89 ± 0.89 mEq/L and 3.22 ± 0.19 mEq/L respectively. The mean value of serum potassium of the control patients is 4.37 ± 0.53 mEq/L which is more than that of intermittent asthmatic patients and persistent asthmatic patients. The reference range is 3.5 – 5.1 mEq/L. The mean of serum potassium levels of intermittent is found to be closer to the control groups mean serum potassium than the persistent groups mean of serum potassium hence better. The association between the two groups was found to be statistically significant. Whyte KF reported the presence of decreased serum potassium in asthmatic patients.[10]

Whang et al. reported that when magnesium is depleted, side by side there is a corresponding decrease in serum potassium which is caused by the decreasing serum magnesium in impairing Na/K ATPase activity, along with increased efflux through K channels resulting in potassium loss through urine.[92] The mean values of Serum Magnesium of intermittent and persistent asthmatic patients is 1.44 ± 0.15 and 1.32 ± 0.62 mg/dl respectively. The mean value of serum magnesium for the control group is 1.96 ± 0.22 mg/dl. The normal reference range for serum Magnesium is 1.7-2.4 mg/dl. The mean serum magnesium of the intermittent group is found to be closer to the control group than the persistent group. This is statically significant also. All of the 44 asthmatic patients had hypomagnesaemia. Alamoudi OS reported that hypomagnesemia and hypophosphatemia are the two most common electrolyte disturbed in asthma.[93]

The mean values of Serum Calcium among the intermittent and persistent asthmatic patients is 7.7 ± 0.67 mg/dl and 8.01 ± 0.74 mg/dl. Statistically there is significance when intermittent and persistent asthmatic group is compared with the control group individually. Although statistically insignificant, hypocalcaemia was seen in both the groups. The reference range being 8.6-10.3 mg/dl. Healthy subjects administered with IV β_2-agonists were reported with Hypocalcemia with an increase in the urinary excretion of calcium.[94]

The mean values of Serum Phosphorus among the intermittent and persistent asthmatic patients is 2.29 ± 0.1 mg/dl and 2.29 ± 0.27 mg/dl respectively. The mean of the control group was 4.24 ± 0.57 mg/dl. The mean values of the control group has significance to the mean values of the persistent asthmatic patient group and intermittent patient groups individually. The asssociation was insignificant among the asthmatic groups. The reference range being 2.5-4.5 mg/dl. Alamoudi OS reported that hypomagnesemia and hypophosphatemia are the two most common electrolyte disturbed in asthma.[93]

A study in Emergency medical services of Denver General Hospital with 23 patients with a history of asthma or chronic pulmonary disease showed that aggressive administration of nebulized Salbutamol during emergency treatment of acute bronchospasm was associated with significant decreases in serum Potassium, Magnesium and Phosphorus.[95]

Another study done at Calcutta National Medical College, Kolkata with 50 asthmatic patients selected randomly who were attending the outpatients department of respiratory medicine showed 14 having hypomagnesaemia with

tachypnoea who used long acting beta agonist, inhaled corticosteroids.[96]

A study done on 60 asthmatic patients in Adichunchanagiri Institute of Medical sciences and Hassan Government Medical college, Karnataka on their serum electrolytes levels during nebulised salbutamol therapy showed that serum electrolytes like magnesium, potassium and phosphorus decreased significantly in patients with acute severe asthma who were on treatment with nebulised salbutamol.[97]

CONCLUSION:

The study was done to evaluate the electrolytes in asthmatic patients who were attending SMIMS. The parameters that were taken into consideration were serum sodium, potassium, magnesium, phosphorus and calcium. As expected there was derangement of serum electrolytes in the asthmatic patient such as Hypokalemia, Hypomagnesaemia, Hyponatremia, and Hypophosphatemia. The

asthmatic patients showed electrolyte abnormality in combinations. The study also showed the probable existence of hypocalcemia (below 8.5mg/dl) which was not statistically significant and probably warrants the need for a study involving a larger sample population to verify the significance if any. A similar finding of insignificance is to be noted with respect to hypernatremia. The population of the study were people from in and around Kulasekharam a place known to have plenty of rubber trees and stone factories providing the aetiology for a probable dust induced or allergen induced bronchial asthma.

Various studies suggested the existence of serum electrolytes abnormality as a single entity or in combinations. This study was done to compare the electrolyte abnormality among the intermittent and persistent asthmatic cases with the hypothesis that as the intermittent patients required occasional medication their electrolytes would be better when compared with the persistent asthmatic group. The study showed that asthmatic patients presented with Hyponatremia, Hypokalemia, Hypomagnesaemia, Hypocalcemia or Hypophophatemia. The association of hypomagnesium and hypokalemia was seen strongly in asthmatic patients. Between the asthmatic groups serum potassium, serum sodium and serum magnesium of the intermittent asthmatic group is found to be better compared to the persistent group.

Summary:

Bronchial asthma is a common respiratory disease caused due to an airway inflammation following with difficulty in breathing normally. It is caused by many factors. Treatment involves in alleviating the initial symptoms. Beta agonists is used as the first line of drug to treat asthma, which is known to cause abnormality in the serum electrolytes.

The present case – control study involved 44 asthmatics as cases. Based on GINA classification for Asthma the asthmatic patients were grouped as Intermittent and persistent and compared with the control group's serum electrolyte. The serum electrolyte which were considered for this study was sodium, potassium, magnesium, calcium and phosphorus. The cases were found to have various abnormalities such as Hypokalemia, Hypomagnesaemia, Hyponatremia, Hypophosphatemia and Hypocalcemia. The serum electrolyte in this study among the cases showed 3 electrolytes were better to persistent which could be statistically proved significant.

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ANNEXURES

CONSENT FORM

PART – II

The details of the study have been explained to me in writing and the details have been fully explained to me. I am aware that the results of the study may not be directly beneficial to me but will help in the advancement of medical sciences. I confirm that I have understood the study and had the opportunity to ask questions. I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without the medical care that will normally be provided by the hospital being affected. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). I have been given an information sheet giving details of the study. I fully consent to participate in the study "A study of serum electrolytes abnormality in Asthmatics". I give consent for withdrawing blood (5ml) from my body for your study.

Serial no/Reference no:

Name of the Participant: **Address of the Participant.**

Contact number of the Participant:

Signature/Thumb impression of the participant/Legal guardian

Witness

1.

2.

Date:

Place: Kulasekharam

CASE PROFORMA
SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES
KULASEKHARAM

TITLE OF STUDY: "A study of serum electrolytes abnormality in Asthmatics"

HOSPITAL NO :
SERIAL NO :
DATE :
NAME :
AGE :
SEX :
Occupation :
ADDRESS AND PHONE NO :

BRIEF HISTORY :
PRESENT C/O :
DURATION OF ASTHMA:
FREQUENCY OF ATTACKS:
LAST ATTACK :

TREATMENT HISTORY :
CURRENT MEDICATION:
DURATION OF USAGE:
ROUTE OF ADMINISTRATION:
FREQUENCY OF USE:
ANY OTHER MEDICINES FOR OTHER ILLNESS:

GENERAL EXAMINATION:
PULSE: BP: RESPIRATORY RATE:

INVESTIGATION: Serum Potassium, Magnesium, Calcium, Phosphate and Sodium

SIGNATURE OF INVESTIGATOR

Sree Mookambika Institute of Medical Sciences
Kulasekharam (K.K District, TN) 629161

Phone No: 04651-280866, Fax No. 04651-280740

Institutional Human Ethics Committee

Registered under CDSCO with Reg No. ECR/446/Inst/TN/2013

Ref. No. SMIMS/IHEC/2013/C/08 **Date: 27th December 2013**

Certificate

This is to certify that the Research Protocol Ref. No. **SMIMS/IHEC/2013/C/08,** entitled "A Study of Serum Electrolytes Abnormality in Asthmatics" submitted by Dr. J. Aaron V. Jose, Postgraduate of Department of Biochemistry, SMIMS has been approved by the Institutional Human Ethics Committee at its meeting held on 19th of December 2013.

[This Institutional Human Ethics Committee is organized and operates according to the requirements of ICH-GCP/GLP guidelines and requirements of the Amended Schedule-Y of Drugs and Cosmetics Act, 1940 and Rules 1945 of Government of India.]

SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES * KULASEKHARAM * IHEC Date 27/12/2013

Dr. Rema Menon. N
Member Secretary
Institutional Human Ethics Committee
Professor of Pharmacology and HOD
SMIMS, Kulasekharam [K.K District]
Tamil Nadu -629161

CERTIFICATE

We the members of the Research committee have screened the protocol of the dissertation submitted by the P.G. Students ..Dr. T. AARON B. JOSE (Biochemistry).. in detail and found itself to be fit enough for submitting to the IHEC for approval.

2/12/13

Chairperson

Dr. Haneephabi.

Professor of Community Medicine

Kumari Sheela MS
2-12-13

Convenor

Dr. M.S. Kumari Sheela MD.

Professor & HOD Physiology

Members

1) Mr. M.B. Kumar - Statistician
2) Dr. Rema Menon - Professor & HOD Pharmacology
3) Dr. Pethuru - Epidemiologist
4) Dr. Kaniraj Peter – Professor of Medicine
5) Dr. Balachandran - Professor of OBG
6) Dr. Sreelal - Professor dental College.

MASTER CHART CONTROL

	Name	Age	Sex	Sodium	Potassium	Magnesium	Calcium	Phosphorus
1	ALI FATHIMA	43	2. Female	135	3.7	2.5	8.6	4.5
2	ANUSHA	25	2. Female	137	4.1	2	9	4.5
3	SHOBA	41	2. Female	138	4.2	1.7	8.9	4.4
4	JENNIFER	19	2.Female	136	3.8	2	8.9	4.3
5	LATHIKA	31	2. Female	139	4.6	1.8	7.2	4.4
6	SUBIN RAJ	32	1. Male	136	4.2	1.8	8.7	2.5
7	SELVAN	34	1. Male	138	5.6	1.9	7.7	4.6
8	JUSTIN	40	1. Male	143	4.6	2.1	8.5	4.5
9	SASI KALA	58	2. Female	143	3.6	2	8.4	3
10	BABU	50	1. Male	135	3.7	1.7	9	5.1
11	PUORUSOTHAMAN	60	1. Male	132	4	2	9.2	4.4
12	ACHUTHAN	54	1. Male	131	4.5	1.8	8.1	4.5
13	MOHAN	54	1. Male	136	5.1	2.4	8.5	4.5
14	DOMINIC XAVIER	55	1. Male	135	5	1.8	7.8	4.2
15	ROSE MARY	56	2. Female	135	4.1	2.5	9.9	4.8
16	SELBERI	33	1. Male	137	4.7	1.9	8.3	4.2
17	SASI KUMAR	48	1. Male	138	5	2.4	9.4	4.6
18	MANIKANDAN	25	1. Male	136	4.2	2	7.2	3.7
19	SIVA KAMI	21	2. Female	137	4	2.3	9.9	4.4
20	ANDREW JOBISH	41	1. Male	133	4.8	2.1	9.5	4.2
21	MARY PUSHPABAI	52	2. Female	136	5.1	1.9	8.4	3.4

22	VELAMMA	60	2. Female	141	3.5	2.1	9.1	4.8
23	KRISHNAN	54	1. Male	135	4	1.8	7.9	3.6
24	VIJAYA KUMAR	30	1. Male	140	5.1	1.9	8.4	4.8
25	ALFRED	37	1. Male	134	5	2	8.2	3.8
26	JEBA PRAKASH	48	1. Male	138	5.3	1.7	7.3	4
27	SEKHAR	53	1. Male	136	4.9	1.8	8.6	4.1
28	PICHANDIPILLAI	60	1. Male	136	4.7	1.8	7.9	3.9
29	SOMY	32	2. Female	137	3.7	1.9	8.5	3.9
30	CHINCHU	25	2. Female	139	4.2	1.7	9	5.1
31	FLIPPO	34	1. Male	137	4.9	1.9	9.8	4.4
32	THAPASIMUTHU	59	1. Male	137	4.6	1.6	9.1	5
33	REMYA	23	2. Female	138	4.2	1.7	8.6	4
34	SENTHIL	42	1. Male	137	4	1.8	9.2	4
35	THANKA RAJ	43	1. Male	136	5.1	1.9	8	4.2
36	SUBBURAM	54	1. Male	138	3.9	2.1	7.4	4.4
37	AMARAVATHY	55	2. Female	137	3.9	1.7	7.4	4.4
38	VILASINI	56	2. Female	135	3.9	1.9	8.1	4.4
39	RAJAM	50	2. Female	137	4.3	1.8	8.5	3.5
40	RAGAVAN NAIR	55	1. Male	134	3.9	2.1	8.6	4
41	BENSAR	59	1. Male	130	3.9	2.2	8.7	2.9
42	CHANDRASEKHARAN	48	1. Male	138	4.1	2.1	8.8	5
43	RAMAR	54	1. Male	135	5	2.2	8.9	5
44	DAISY	58	2. Female	137	4	2.1	9.1	4.7

MASTER CHART CASES

Date	Name	Age	Sex	Sodium	Potassium	Magnesium	Calcium	Phosphorus	Intermittent / persistent	<1symptom per week	<2 nocturnal symptoms
1	KUMAR	48	1.Male	136	4.6	1.5	7.7	2.2	i	1.Yes	1.Yes
2	MANIKANDAN	19	1. Male	134	6.3	1.5	7.6	2.4	i	1. Yes	1. Yes
3	SURENDRAN	60	1. Male	134	3.1	4	6.6	2.3	p	2. No	2. No
4	ASIA	20	2. Female	135	3.5	1.3	7.6	2.3	i	1. Yes	1. Yes
5	VIDHYA	32	2. Female	135	4.3	1	7.2	2.4	i	1. Yes	1. Yes
6	ESSAKI RAJ	26	1. Male	135	4.9	1.5	8.4	2.1	i	1. Yes	1. Yes
7	SULAIKA BEEVI	56	2. Female	133	4.6	1.4	8.4	2.4	i	1. Yes	1. Yes
8	JOHNSON	60	1. Male	134	5	1.3	7.6	2.3	i	1. Yes	1. Yes
9	NASZEERA	46	2. Female	130	3.7	1.1	7.9	3.4	p	2. No	2. No
10	ALEX	18	1. Male	134	5.1	1.5	8.2	2.2	i	1. Yes	1. Yes
11	SHINY	34	2.Female	134	3.1	1.5	8.3	2.3	p	2.No	2.No
12	THANKAMMAL	58	2. Female	133	4.8	1.6	8.1	2.3	i	1. Yes	1. Yes
13	KUMARI JEYA	52	2. Female	134	3.1	1.5	6.7	2.3	i	1. Yes	1. Yes
14	THANKAMMA	53	2. Female	135	3.1	1.2	6.8	2.4	i	1. Yes	1. Yes
15	MINI THANKAM	45	2. Female	135	3.2	1.4	8.3	2.2	i	1. Yes	1. Yes
16	KRISHNAN	54	1. Male	131	2.7	1.2	5.8	2.1	p	2. No	2. No
17	KANNAN	54	1. Male	130	3.1	1.2	6.6	2.4	p	2. No	2. No
18	POOMANI	42	2. Female	134	3.4	1.4	7.6	2.3	i	1. Yes	1. Yes
19	SAMUVEL	47	1. Male	134	3.3	1.3	8.1	2.4	i	1. Yes	1. Yes
20	RADHA NAIR	54	2. Female	135	3.1	1.6	6	2.4	i	1. Yes	1. Yes
21	NESAYYAN	60	1. Male	135	3.4	1.5	8.4	2.2	i	1. Yes	1. Yes

22	ESWARAPRASAD	57	1. Male	136	3.4	1.5	7.3	2.1	i	1. Yes	1. Yes
23	MERLIN	26	2. Female	134	3.3	1.6	6.8	2.1	i	1. Yes	1. Yes
24	GANAPATHY	58	1. Male	135	3.2	1.4	8.3	2.3	i	1. Yes	1. Yes
25	JOHN PAUL	30	1. Male	136	3.3	1.6	7.6	2.3	i	1. Yes	1. Yes
26	THANKA RAJ	50	1. Male	134	3.4	1.5	8.4	2.4	i	1. Yes	1. Yes
27	ELIZABETH	48	2. Female	136	3.4	1.6	8.3	2.4	i	1. Yes	1. Yes
28	SUBRAMANIAN	48	1. Male	132	3.1	1.2	8.5	2.1	p	2.No	2.No
29	GOBINATH	45	1. Male	132	3.3	1.3	8	2.4	p	2.No	2.No
30	GOMATHY	53	2. Female	134	3.3	1.4	8.1	2.3	p	2.No	2.No
31	ABDUL WAHAB	55	1. Male	134	3.4	1.4	8.4	2.2	p	2.No	2.No
32	GEETHA	18	2. Female	131	3.2	1	8.5	2.1	p	2.No	2.No
33	PAPPA	45	2. Female	134	3.2	0.8	8.3	2.4	p	2.No	2.No
34	SOUNDARA BAI	56	2. Female	134	3.3	0.9	8.2	2.3	p	2.No	2.No
35	FELSITAL MARY	48	2. Female	133	3.2	1.3	8.1	2.2	p	2.No	2.No
36	SUSHMITHA	21	2. Female	128	3.1	1.4	8.5	2.1	p	2.No	2.No
37	RENY JOY	29	2. Female	132	3.2	1.2	8.6	2.1	p	2.No	2.No
38	KARTHIKA	23	2. Female	134	3.1	1.1	8.3	2.4	p	2.No	2.No
39	RENJITH	32	1. Male	133	3.4	1.3	8.8	2.3	p	2.No	2.No
40	SHYMA	37	2. Female	135	3.4	1.1	8.2	2.2	p	2.No	2.No
41	RAVI CHANDRAN	41	1. Male	135	3.4	1.1	8.5	2.1	p	2.No	2.No
42	CHELLATHAI	60	2. Female	136	3.3	1.3	7.5	2.3	p	2.No	2.No
43	RAJENDRAN	41	1. Male	132	3.2	1.3	8.3	2.2	p	2.No	2.No
44	LOBISAL	59	2. Female	132	3.1	1	8.3	2.2	p	2.No	2.No